FORMULATION AND *IN VITRO* EVALUATION OF LIPOSOMAL DRUG DELIVERY SYSTEM OF METFORMIN HCL

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THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

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In partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY

IN

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Submitted by

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DEDICATED TO ALMIGHTY GOD, OUR BELOVED PARENTS AND TEACHERS

ABBREVIATIONS

NDDS	=	Novel Drug Delivery system
HCl	=	Hydro chloride
nm	=	Nanometer
µm	=	Micrometer
R.E.S	=	Reticulo endothelial system
BBB	=	Blood Brain Barrier
SUVS	=	Small unilamellar vesicles
DNA	=	Deoxyribonucleic acid
DEAE	=	Diethylaminoethyl cellulose
MLV	=	Multilamellar Large Vesicles
UV	=	Unilamellar vesicles
MUV	=	Medium sized unilamellar vesicles
(LUV	=	Large unilamellar vesicles
GUV	=	Giant unilamellar vesicles
OLV	=	Oligolamellar vesicles
MVV	=	Multivesicular vesicles
LET	=	Liposomal encapsulation technology
REV	=	Reverse phase evaporation vesicles
CMC	=	Critical micelle concentration
UV	=	Ultra violet spectrometer
pH	=	Potential of hydrogen
FT-IR	=	Fourier transform infrared spectroscopy
IV	=	Intravenous
GIT	=	Gastro intestinal tract
%	=	Percentage

CONTENT

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INTRODUCTION

Novel Drug Delivery System:

Novel Drug Delivery system (NDDS) refers to the approaches, formulations, technologies, and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effects. NDDS is a system for delivery of drug other than conventional drug delivery system. NDDS is a combination of advance technique and dosage form which are far better than conventional dosage form[1].The aim of NDDS is to provide a therapeutic amount of drug to the appropriate site in the body to accomplish promptly and then maintain the desired drug concentration[2]. NDDS combining polymer science, pharmaceutics and molecular biology[3].

ADVANTAGES OF NOVEL DRUG DELIVERY SYSTEM[4, 5]:

i. Optimum dose at the right time and right location

ii. Efficient use of expensive drugs, excipients and reduction in production cost

iii. Improves the therapy by increasing the duration of action and reducing the side effects.

iv. Increases the patient compliance and provides convenient route of administration.

v. Achieve the targeting of drugs to a specific sites which reduces the unwanted side effects and obtain maximum efficacy.

vi. Reduces the dose and thus reduces the side effects of drugs.

Types of novel drug delivery systems[4,5]:

There are number of novel drug delivery systems are available. They are

1. Hydrogels

2. Colloidal drug carrier systems

a) Micelles

b) Microspheres

c) Nanoparticles

d) Liposomes and neosomes

3. Mucoadhesives

4. Transdermal drug delivery

5. Ocular drug delivery

6. Nasal drug delivery

1. Hydrogels:

Hydrogels are three dimensional hydrophilic polymeric networks capable of absorbing large amount of water or biological fluids. These networks are composed of homopolymers or copolymers and are insoluble because of the presence of chemical or physical crosslinks like entanglements or crystallites. The hydrogels exhibit thermodynamic compatibility with water which allows them to swell in aqueous medium. They are used to control the drug release in reservoir based controlled release system or as carriers in swellable and swelling control release devices[4].

2. Colloidal Drug Carrier Systems:

Colloidal drug carrier systems like micellar solutions, vesicle and liquid crystal dispersions, microspheres, nanoparticles, consisting of small particles, ranging from 10 nm to 400 nm diameter. They show great promise as drug delivery systems. When developing these formulations the aim is to obtain systems with optimized drug loading and release properties, long shelf life and low toxicity[4].

a) **Micelles:**

Micelles formed by the self-assemble of amphiphilc block copolymers in aqueous solutions. The size ranges from 5 to 50 nm. They will provide grate interest in drug delivery applications. The drugs can be physically entrapped in the core of block

co polymer micelle and transported at concentration that can exceed their intrinsic water solubility[4].

b) Microspheres:

Microspheres are the delivery systems that contain a matrix of the polymer in which the drug in micron size is uniformly dispersed[5]. It comprises of small spherical particles, with diameters in the micrometer range, typically 1µm to 1000µm[5]. Microcapsules are those where the drug is coated with the polymer[4]. The microcapsules and microspheres prolong drug release whereas microspheres are used for drug targeting[4].

c) Nanoparticles:

The size ranges from 10 to 1000 nm. They can absorb and encapsulate a drug thus protecting it from chemical and enzymatic degradation. The nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a unique polymer membrane. Nanospheres are matrix systems in which the drug is physically and uniformly dispersed. Nanoparticles as drug carriers will be formed from both biodegradable and non-biodegradable polymers. They will provide massive advantages regarding drug targeting, delivery, and release[4]. Especially, used for the delivery of lipophilic compounds[5].

d) Liposomes and Niosomes:

Liposomes are concentric bi-layered vesicles in which aqueous volume is entirely enclosed by a membranous lipid bi-layer mainly composed of natural or synthetic phospholipids. The liposomes are spherical particles that encapsulate the solvents which are freely floating in the interior[5]. Amphiphillic and lipophilic molecules are solubilised with in the phospholipid bi layer according to their affinity towards phospholipids. Presence of non-ionic surfactant instead of phospholipids in the formation of bilayers results in the formation of neosomes[4].

3. Mucoadhesive Systems:

Mucoadhesives are synthetic or natural polymers that interact with the mucus layer covering the mucosal epithelial surface and mucin molecules. They can adhere to the gastric mucosa or the buccal mucosa. This concept has altered the possibility that these polymers can be used to overcome physiological barriers in long term drug

delivery. This mucoadhesive drug delivery system gives more effective and safe treatment not only for topical disorders but also for systemic problems[4].

4. Transdermal Drug Delivery:

Transdermal drug delivery is defined as self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug, through the skin at controlled rate to the systemic circulation[6]. If the skin is the site of action then high concentration of drugs can be localized at the skin, which results in reducing the systemic drug levels and also reducing the systemic side effects. It is an alternative route for the delivery of systemically acting drugs. This route have several advantages when compared with oral drug administration. It bypasses the lever there by the dose is reduced and the side effects are minimized[4].

5. Ocular Drug Delivery:

Ocular drug delivery is the one of the most challenging drug delivery system. This field has improved significantly over the past 20 years. The improvements have largely focused on maintaining the drug in eyes for an extended period of time unlike conventional eye drops[4].

6. Nasal Drug Delivery:

The nasal route appears to be an alternative to parenterals for administrating drugs intended for systemic effects. The nasal route provides rich vascularity high permeable structure for absorption. It avoids hepatic first pass metabolism. Proteins such as insulin are reported to have fast and sustained action when administered through the nasal route[4].

LIPOSOMES-An Introduction

Liposomes are colloidal, vesicular structure composed of one or more bilayers surrounding an equal number of aqueous compartment[7]. Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural nontoxic phospholipids. Due to their size and hydrophobic and hydrophilic character (besides biocompatibility), liposomes are promising systems for drug delivery[8]. The sphere like shell encapsulated a liquid interior which contain substances such as peptides, protein, hormones, enzymes, antibiotics, anti-fungal and anti-cancer agents[7].

Liposome properties differ considerably with lipid composition, surface charge, size, and the method of preparation. Furthermore, the choice of bilayer components determines the 'rigidity' or 'fluidity' and the charge of the bilayer. For instance, unsaturated phosphatidylcholine species from natural sources (egg or soybean phosphatidylcholine) give much more permeable and less stable bilayers, whereas the saturated phospholipids with long acyl chains (for example, dipalmitoylphosphatidylcholine) form a rigid, rather impermeable bilayer structure[8].

It has been displayed that phospholipids impulsively form closed structures when they are hydrated in aqueous solutions. Such vesicles which have one or more phospholipid bilayer membranes can transport aqueous or lipid drugs, depending on the nature of those drugs. Because lipids are amphipathic (both hydrophobic and hydrophilic) in aqueous media, their thermodynamic phase properties and self-assembling characteristics influence entropically focused confiscation of their hydrophobic sections into spherical bilayers. Those layers are referred to as lamellae[9].

Liposomes particle sizes ranges from 30 nm to several micrometers. They consist of one or more lipid bilayers surrounding aqueous units, where the polar head groups are oriented in the pathway of the interior and exterior aqueous phases. On the other hand, self-aggregation of polar lipids is not limited to conventional bilayer structures which rely on molecular shape, temperature, and environmental and preparation conditions but may self-assemble into various types of colloidal particles[10].

Advantages of Liposomes:

Some of the advantages of liposome are as follows[1,8,11, 12]:

1) It can carry both water and lipid soluble drugs.
2) Provides selective passive targeting to tumor tissues (liposomal doxorubicin).

3) Liposomes increased efficacy and therapeutic index of drug (actinomycin-D).
4) Liposome increased stability via encapsulation.
5) Liposomes are non-toxic, flexible, biocompatible, completely biodegradable, and non-immunogenic for systemic and non-systemic administrations.
6) Liposomes reduce the toxicity of the encapsulated agent (amphotericin B, Taxol).
7) Liposomes help reduce the exposure of sensitive tissues to toxic drugs.
8) Site avoidance effect.
9) Flexibility to couple with site-specific ligands to achieve active targeting.
10) Improved pharmacokinetic effects (reduced elimination, increased circulation life times).
11) It provide sustained release.
12) It can be administered through various routes.
13) It engenders incorporate micro and macro molecules.
14) It also act as reservoir of drugs.
15) Liposomes can modulate the distribution of drug.
16) It direct interaction of the drug with cell.

Disadvantages of Liposomes:

Some of the disadvantages of liposome are as follows[8,11]:

1) Low solubility.
2) Sometimes phospholipid undergoes oxidation and hydrolysis-like reaction.
3) Short half-life.
4) Leakage and fusion of encapsulated drug/molecules.
5) Production cost is high.
6) Fewer stables.
7) Quick uptake by cells of reticuloendothelial system (R.E.S).
8) Allergic reactions may occur to liposomal constituents.
9) Problem to targeting to various tissues due to their large size.

Application of Liposomes:

Liposomes for Brain Targeting:

The biocompatible and biodegradable behavior of liposomes have recently led to their exploration as drug delivery system to brain. Liposomes with a small diameter (100 nm) as well as large diameter undergo free diffusion through the Blood Brain

Barrier (BBB). However it is possible that a small unilamellar vesicles (SUVS) coupled to brain drug transport vectors may be transported through the BBB by receptor mediated or absorptive mediated transcytosis[13].

Liposome in Eye Disorders:

Liposome has been widely used to treat disorder of both anterior and posterior segment. The disease of eye includes dry eyes, keratitis, corneal transplant rejection, uveitis, endopthelmitis and proliferative vitro retinopathy. Retinal diseases are leading cause of blindness in advanced countries. Liposome is used as vector for genetic transfection and monoclonal antibody directed vehicle. The recent techniques of the treatment like applying of focal laser to heat induced release of liposomal drugs and dyes are used in the treatment of selective tumor and neo-vascular vessels occlusion, angiography, retinal and choroidal blood vessel stasis[13].

Liposome for Respiratory Drug Delivery System:

Liposome is widely used in several types of respiratory disorders. The recent use of liposome for the delivery of DNA to the lung means that a greater understanding of their use in macromolecular delivery via inhalational is now emerging. Much of this new knowledge, including new lipids and analytical techniques, can be used in the development of liposome based protein formulations. For inhalation of liposome the liquid or dry form is taken and the drug release occurs during nebulization. Drug powder liposome has been produced by milling or by spray drying[13].

Liposomes in parasitic diseases and infections:

Since conventional liposomes are digested by phagocytic cells in the body after intravenous administration, they are ideal vehicles for the targeting of drug molecules into these macrophages. The best known examples of this 'Trojan horse-like' mechanism are several parasitic diseases which normally reside in the cell of mononuclear phagocytic system. They include leishmaniasis and several fungal infections[14].

Macrophage activation and vaccination:

Some natural toxins induce strong macrophage response which results in macrophage activation. This can be duplicated and improved by the use of liposomes because small molecules with immunogenic properties (haptens) cannot induce

immune response without being attached to a larger particle. For instance, liposomes containing muramyl tripeptide, the smallest bacterial cell wall subunit with immunogenic properties cause macrophage activation. Activated macrophages are larger and contain more granulomae and lysosome material. Their state lasts for a few days during which they show enhanced tumouricidal, virocidal and microbicidal activity[14].

Liposomes in anticancer therapy:

Many different liposome formulations of various anticancer agents were shown to be less toxic than the free drug. Anthracyclines are drugs which stop the growth of dividing cells by intercalating into the DNA and therefore kill predominantly quickly dividing cells. These cells are in tumours, but also in gastrointestinal mucosa, hair and blood cells and therefore this class of drugs is very toxic[14].

Liposomes in bioengineering:

Nucleic acids used in gene transfer are large, with molecular weights up to several million Daltons, highly charged and hydrophilic and therefore not easy to transfer across cell membranes. Additionally to classical methods, such as direct injection, phosphate precipitation and others, liposomes were tried as transfection vectors. They can deliver the encapsulated or bound nucleic acid into cells predominantly in two ways: the classical approach is to encapsulate the genetic material into liposomes and liposomes act as an endocytosis enhancer while recently the phosphate or DEAE precipitation was simulated by liposomes. In these cases the nucleic acid forms a complex with several cationic liposomes and the size of the complex and its adsorption on the cell surface catalyses endocytosis or, possibly, fusion[14].

Liposomes in cosmetics:

Liposomes as a carrier itself offers advantages because lipids are well hydrated and can reduce the dryness of the skin which is a primary cause for its ageing. Also, liposomes can act to replenish lipids and, importantly, linolenic acid[14].

Liposomes in agro-food industry:

Lipid molecules from fats to polar lipids, are one of the fundamental ingredients in almost any food. The sustained release system concept can be used in

various fermentation processes in which the encapsulated enzymes can greatly shorten fermentation times and improve the quality of the product. This is due to improved spatial and temporal release of the ingredient(s) as well as to their protection in particular phases of the process against chemical degradation. A classical example is cheese making[14].

Mechanism of Liposome Formation[15]:

In aqueous medium, the lipid molecules in self-assembled structures are oriented in such a way that the polar portion of the molecule remains in contact with the polar environment and at the same time shields the non-polar part. Among the amphiphiles used in the drug delivery, viz. soap, detergents, polar lipids, the latter (polar lipids) are often employed to form concentric bilayered structures. However, in aqueous mixtures these molecules are able to form various phases, some of them are stable and others remain in the metastable state. At high concentrations of these polar lipids, liquid-crystalline phases are formed that upon dilution with an excess o water can be dispersed into relatively stable colloidal particles.

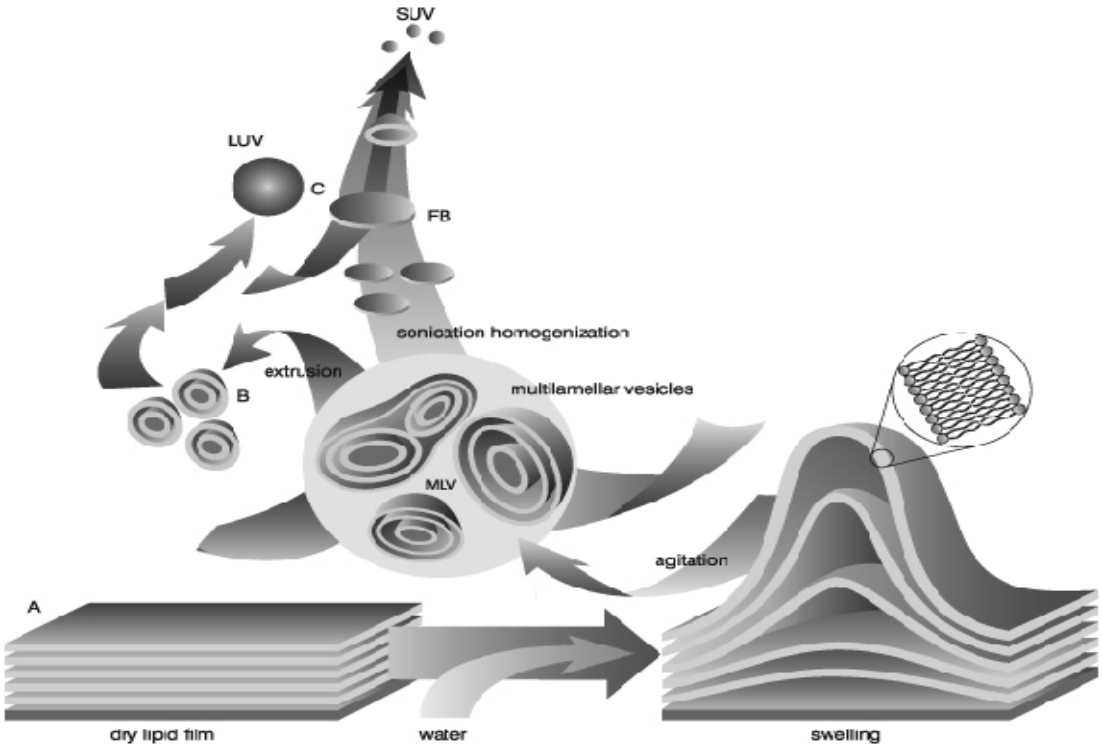


Figure 1: Mechanism of liposome formation

Classification of liposomes:

Liposome classification based on structural features

Multilamellar Large Vesicles (MLV):

In MLV, vesicles have an onion structure. Classically, several unilamellar vesicles will form on the inside of the other with smaller size, making a multilamellar structure of concentric phospholipid spheres separated by layers of water[8].

Unilamellar vesicles (UV):

In UV liposomes, the vesicle has a single phospholipid bilayer sphere enclosing the aqueous solution. UV vesicles can be prepared in a variety sizes[16]:

Small unilamellar vesicles (SUV) - 20 to 40 nm.

Medium sized unilamellar vesicles (MUV) – 40 to 80 nm.

Large unilamellar vesicles (LUV) – 10 to 1000 nm.

Giant unilamellar vesicles (GUV) - > 1000 nm.

Oligolamellar vesicles (OLV):

OLV have large central aqueous cores surrounded by 2 to 10 bilayers[16].

Multivesicular vesicles (MVV):

MVV first described as large clusters of smaller compartments sharing common bilayers, have been redefined to cover all structures of non-concentric vesicles inside a larger vesicle of 200 nm to 3μm[17].

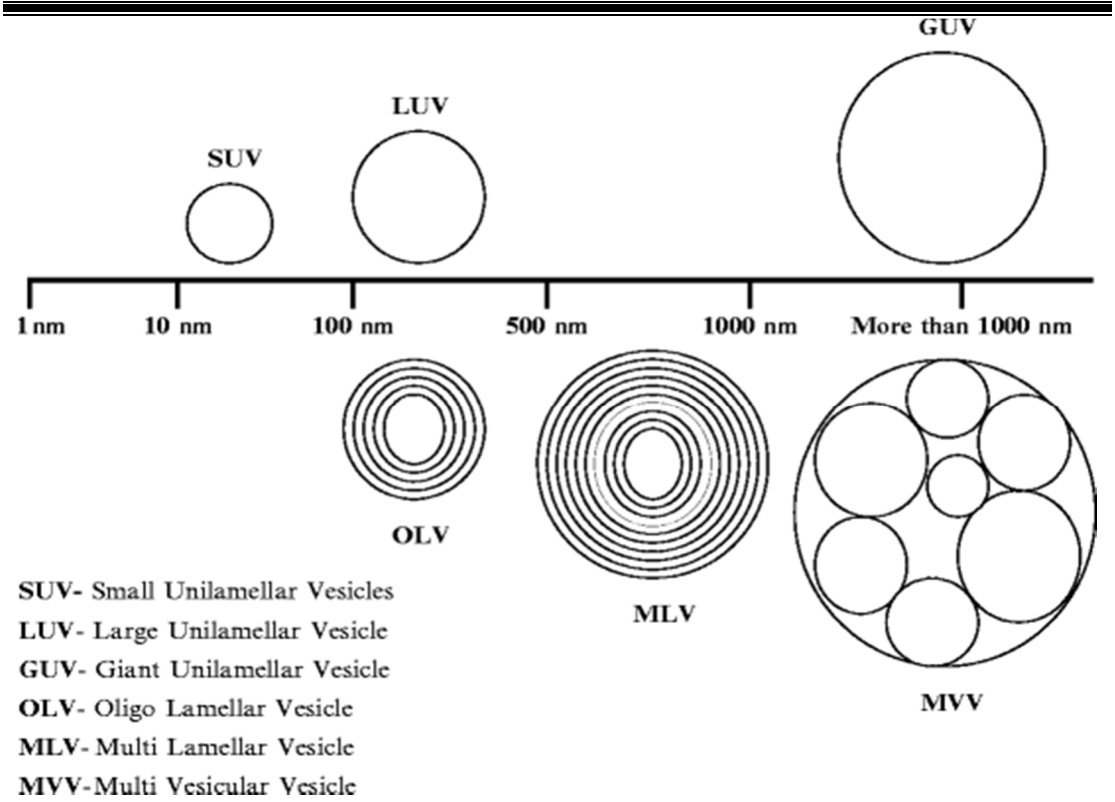


Figure 2: Classification of liposome formation

Methods of Liposomes Preparation:

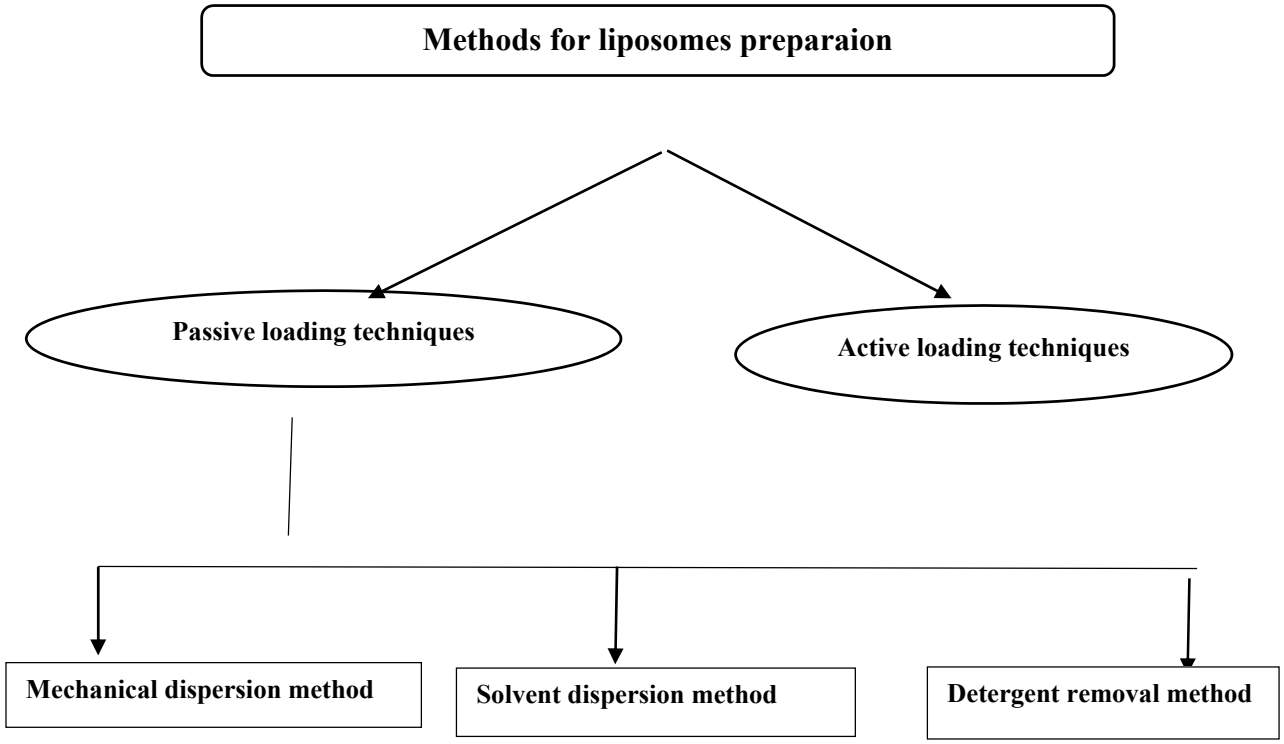


Figure No. 3 Methods of liposome preparation

A. Passive loading techniques:

In these passive loading technique the drug is encapsulated by incorporating an aqueous phase of a water-soluble (hydrophillic) drug or an organic phase of a lipid-soluble drug initially or at predetermined stage during the preparation of the liposomes. The huge drug encapsulation efficiency can be achieved with the help of these passive

loading technique which is more suitable for lipid-soluble drugs with a high resemblance to the lipid membrane.

Different methods discuss under this class start with a lipid solution in organic solvent and nd up with lipid dispersion in water. The a choice of component are typically combined by co-dissolving the lipids in an organic solvent and the organic solvent is then seperated by film deposition under vacuum .When residual solvent is removed, the solid lipid mixture is hydrated with the help of aqueous buffer.

The lipids spontaneously swell and hydrate to form liposome. Liposomal encapsulation technology (LET) is the latest delivery method used by medical researcher to transmit drugs that act as healing promoters to the definite body organs. LET is state of art method of preparing sub-microscopic bubbles called liposome[18].

1. Mechanical Dispersion Method:

In these method variety component are mainly combined by co-dissolving the lipids in an organic solvent and after that the organic solvent is then separated by film deposition under vaccum. When all the solvent is evaporated, the solid lipid mixture is hydrated using aqueous phase. The lipids spontaneously swell and hydrate to form liposomes[18].

The following are types of mechanical dispersion methods:

i. Lipid film hydration method:

The lipid-film hydration procedure is the most common and simple method for preparation of MLV by dissolving the phospholipids in the organic solvents: dichloromethane, chloroform, ethanol and chloroform-methanol mixture (2:1 v/v; 9:1 v/v; 3:1 v/v). A thin and homogeneous lipid film is formed when solvent is evaporated under vacuum at the temperature: 45-60 ºC. Nitrogen gas is involved in order to completely remove the residual solvent. A solution of distilled water, phosphate buffer, phosphate saline buffer at pH 7.4 and normal saline buffer are used in hydration step. The time for the hydration process varied from 1 h to 2 h at the temperature 60- 70 ºC. In order to obtain full lipid hydration, the liposomal suspension is left overnight at 4 ºC. The lipid-film hydration method can be used for all different kinds of lipid mixtures[19].

i. Micro-emulsification method:

An equipment called as microfluidizer is used to prepare small vesicle from concentrated lipid suspension. The lipids can be introduced into the fluidizer as a

suspension of large MLVs. This equipment pumps the suspension at very high pressure through the 5 mm screen. Then it is forced long micro channel, which direct two streams of fluid collide together at right angle and very high velocity. The fluid collected can be recycled through the pump and interaction chamber until vesicles of spherical dimension are obtain[20].

iii. Sonication

Sonication is perhaps the most extensively used method for the preparation of SUV. Here, MLVs are sonicated either with a bath type sonicator or a probe sonicator under a passive atmosphere. The main disadvantages of this method are very low internal volume/encapsulation efficacy, possible degradation of phospholipids and compounds to be encapsulated, elimination of large molecules, metal pollution from probe tip, and presence of MLV along with SUV. There are two sonication techniques[21].

a. Probe sonication: The tip of a sonicator is directly engrossed into the liposome dispersion. The energy input into lipid dispersion is very high in this method. The coupling of energy at the tip results in local hotness; therefore, the vessel must be engrossed into a water/ice bath. Throughout the sonication up to 1 h, more than 5% of the lipids can be de-esterified. Also, with the probe sonicator, titanium will slough off and pollute the solution[21].

b. Bath sonication: The liposome dispersion in a cylinder is placed into a bath sonicator. Controlling the temperature of the lipid dispersion is usually easier in this method, in contrast to sonication by dispersal directly using the tip. The material being sonicated can be protected in a sterile vessel, dissimilar the probe units, or under an inert atmosphere[21]

iv. French pressure cell:

French pressure cell involves the extrusion of MLV through a small orifice. An important feature of the French press vesicle method is that the proteins do not seem to be significantly pretentious during the procedure as they are in sonication. An interesting comment is that French press vesicle appears to recall entrapped solutes significantly longer than SUVs do, produced by sonication or detergent removal. The method involves gentle handling of unstable materials. The method has several advantages over sonication method. The resulting liposomes are rather larger than

sonicated SUVs. The drawbacks of the method are that the high temperature is difficult to attain, and the working volumes are comparatively small (about 50 mL as the maximum)[8].

v. Membrane extrusion:

In this method, MLVs is reduced by passing them through a membrane filter of defined bore size. There are two types of membrane filter. The tortuous bath type and the nucleation track type. The former is used for sterile filtration. In this random bath arises between the criss cross fibres in the matrix. Liposomes that are larger than the channel diameter get struck when one tries to pass them though such membrane. The nucleation track is composed of thin continuous sheet of polycarbonate. They will offer less resistance to passage of liposomes as these consist of straight sided pore holes off exact diameter bored from one side to another. This method can be used to process both LUVs and MLVs[18].

vi. Dried reconstituted vesicles:

In DRV method freeze drying of a dispersion of empty SUVs are to be done and then dispersion of it with the aqueous fluid containing the material to be entrapped. This leads to a hydration of solid lipids in finely reduced sized form. Though, the step of freeze-drying is introduced to freeze and lyophilize a performed SUVs dispersion rather than to dry the lipids from an organic solution. This leads to an ordered membrane structure as compared to random matrix structure, which on addition of water can rehydrate, fuse and reseal to form vesicles with a high encapsulation efficiency. The water soluble hydrophillic materials to be entrapped are added to the dispersion which are empty SUVs and they are dried together, so the material for inclusion is present in the dried precursor lipid before the final step of addition of aqueous medium[18].

vii. Freeze-thawed liposome:

SUVs are rapidly frozen and thawed slowly. The short-lived sonication disperses aggregated materials to LUV. The creation of UV is as a result of the fusion of SUV throughout the processes of freezing and thawing. This type of synthesis is strongly inhibited by increasing the phospholipid concentration and by increasing the ionic strength of the medium. The encapsulation efficacies from 20% to 30% were obtained[8].

2. Solvent Dispersion Method:

In these methods lipids are first dissolved in an organic solution and then brought into contact with aqueous phase containing materials to be entrapped within liposome. At the interface between the organic and the aqueous phases the phospholipids align themselves to form a monolayer, which is important step to form the bilayer of liposome[20].

i. Ether injection (solvent vaporization)[8]:

A solution of lipids dissolved in diethyl ether or ether-methanol mixture is gradually injected to an aqueous solution of the material to be encapsulated at 55°C to 65°C or under reduced pressure. The consequent removal of ether under vacuum leads to the creation of liposomes. The main disadvantages of the technique are that the population is heterogeneous (70 to 200 nm) and the exposure of compounds to be encapsulated to organic solvents at high temperature.

ii. Ethanol injection[8]:

A lipid solution of ethanol is rapidly injected to a huge excess of buffer. The MLVs are at once formed. The is advantages of the method are that the population is heterogeneous (30 to 110 nm), liposomes are very dilute, the removal all ethanol is difficult because it forms into azeotrope with water, and the probability of the various biologically active macromolecules to inactivate in the presence of even low amounts of ethanol is high.

iii. Double emulsion method:

In this process, an active ingredient is initially dissolved in an aqueous phase (w1) which is then emulsified in an organic solvent containing polymer to form a primary w1/o emulsion. This primary emulsion is then mixed in an emulsifier which also consist of aqueous solution (w2) to form a w1/o/w2 double emulsion. The extraction of the solvent leaves microspheres in the aqueous external phase, making it possible to seperate them by filtering or centrifuging[18].

iv. Reverse phase evaporation method:

The lipid mixture is added to a round bottom flask and the solvent is removed under reduced pressure by a rotary evaporator. The system is purged with

nitrogen and lipids are re-dissolved in the organic phase which is the phase in which the reverse phase vesicle will form. Diethyl ether and isopropyl ether are the usual solvents of choice. After the lipids are re-dissolved the emulsion are obtained and then the solvent is removed from an emulsion by evaporation to a semisolid gel under reduced pressure. Phosphate buffer saline or citric-Na_2HPO_4 buffer is added to aqueous phase with aim to improve the efficiency of liposome formulations. The formation of liposomes is allowed by continued rotary evaporation of the organic solvents under vacuum. Non encapsulated material is then removed. The resulting liposomes are called reverse phase evaporation vesicles (REV). This method is used for the preparation of LUV and OLV formulation and it has the ability to encapsulate large macromolecules with high efficiency[13,19].

v. Stable pluri lamellar vesicles:

This method of pluri lamellar vesicle preparation followed by formation of water-in-organic phase dispersion with an excess of lipid which further introduce to drying under continued bath sonication with an irregular stream of nitrogen. SPLVs require a large aqueous core, the common of the entrapped aqueous medium being located in the compartment in between adjacent lamellae. The percent entrapment normally ranges around 30%[18].

3. Detergent Removal Method:

In this method the phospholipids are brought into close contact with the aqueous phase via detergents, which associate with phospholipids molecules. The structures formed as a result of this association are known as micelles. They are composed of several hundreds of component molecules. The concentration of detergent in water at which micelles start to form is called CMC. Below CMC the detergent molecule exist in free solution. As the detergent molecule is dissolved in water at concentration higher than the CMC, micelle form in large amounts. As the concentration of detergent added is increased more amount of detergent is incorparorated into the bilayer, until a point is reached where conversion from lamellar form to spherical micellar form take place. As detergent concentration is further increased, the micelles are reduced in size[20].

i. Dialysis:

Detergents are mainly soluble in both aqueous as well as organic media and there is an equilibrium within the detergent molecules in the water phase, and in the lipid environment of the micelle. The CMC can give an indication to the position of this equilibrium. Upon reducing the concentration of detergent in the whole aqueous phase, the molecules of detergent can be washed away from mixed micelle by dialysis. The action of egg PC with a 2:1 molar ratio of sodium cholate followed by dialysis which lead to the formation of vesicles (100nm). A commercial version of the dialysis system is available under the trade name LIPOPREPTM (Diachema AG, Switzerland)[18].

ii. Column Chromatography:

Phospholipids in the form of either sonicated vesicles or as a dry film, at a molar ratio of 2:1 with deoxycholate form UV of 100nm. Deoxycholate remove using column chromatography .This could be done by the passing the dispersion over a Sephadex G-259 column presaturated by constitutive lipids and preequilibrated using hydrating buffer[18].

iii. Dilution:

Upon dilution of aqueous mixed micellar solution of detergent and phospholipids with buffer, the micellar size and the polydispersity increase fundamentally, and as the system is diluted beyond the mixed micellar phase boundary, a spontaneous transition from poly-dispersed micelles to vesicles occurs[8].

B. ACTIVE LOADING:

The exploitation of liposomes as drug delivery system is encouraged with the advancement of well-organized encapsulation procedures. The membrane from the lipid bilayer is in general impermeable to ions and larger hydrophilic molecules. Ions transport can be synchronized by the ionophores though permeation of neutral and weakly hydrophobic molecule can be inhibited by concentration gradients.

A few weak acid or bases yet, can be transported throughout the membrane because of various transmembrane gradient, such as electric, ionic (pH) or specific salt (chemical potential) gradient. Some method exist for improved incorporation of drugs, including remote (active) loading method which load drug molecules into preformed liposome using pH gradient and potential difference across liposomal membrane.

A concentration variation in proton concentration across the membrane of liposomes can drive the loading of amphipathic molecule[18].

Active loading methods have the following benefit over passive encapsulation Technique

a) It will lead to high encapsulation efficiency and capacity.
b) Using these method leakage of the encapsulated compounds can be reduced.
c) "Bed side" loading of drugs therefore limiting loss of retention of drugs by diffusion, or chemical degradation while storage.
d) These process is flexible for constitutive lipid, as drug is loaded after the formation of carrier unit.
e) It also reduce the safety hazard by avoiding biologically active compounds in the preparation step during dispersion.
f) The transmembrane pH gradient may be occured by various method. Based upon the nature of drug to be encapsulated[18].

AIM AND OBJECTIVE OF THE WORK

The aim of the present study was to formulate Metformin HCl liposomes for a sustained drug delivery system. The liposomes was prepared by two different methods (physical dispersion method and ether injection method) and then it was evaluated for various parameters.

The objective of the study is follows as.

To subjugate inherent defects associated with conventional dosage form of Metformin HCl, by formulating oral Metformin HCl liposomes which have the following advantages.

1. Reduce the dose and dosing frequency.
2. Minimize the side effect.
3. Prolong the action of drug.
4. Provide sustained drug release.
5. Better patient compliance.

PLAN OF WORK

The present work carried out to formulate sustained release Metformin HCl liposomes and it was planned to evaluate the various parameters as outlined below:

- To determine the solubility of Metformin HCL in water, methanol and pH 6.8
- Drug-excipients interaction studies by using FT-IR.
- To formulate Metformin HCl liposomes by using cholesterol and lecithin as encapsulated lipids bilayer in various ratio such as 1:1, 1:2 and 1:3 by two different method namely physical dispersion method and ether injection method.
- To evaluate the prepared liposomes for following parameters:
    i. Drug entrapment efficiency.
    ii. Morphological analysis.
    iii. Particle size analysis.
    iv. *In vitro* drug release studies.
    v. Stability studies.

REVIEW OF LITERATURE

Dina Fathalla *et al*[23]., formulated and evaluated liposomal gels for sustained ocular delivery of latanoprost using two different methods, namely thin film hydration and reverse phase evaporation techniques. The objective of their work was to develop a liposome-based delivery system for the sustained ocular delivery of latanoprost, a prostaglandin analog commonly used in the management of glaucoma. Latanoprost was incorporated into different liposomes that were evaluated using variety of techniques. Selected liposomes were incorporated into different gels and their viscosity and drug release kinetics were evaluated. Optimal liposomal gels were evaluated in vivo in rabbits' eyes for their irritation potential and ability to reduce intraocular pressure. Fourier transform infrared and differential scanning calorimetry studies confirmed the interaction between the drug and different excipients in the vesicles, which resulted in drug encapsulation efficiency ≥ 90%. Drug encapsulation efficiency increased with the drug/lipid ratio and encapsulation efficiency ~98% was obtained at drug/lipid ratio of 50%. Vesicles incorporated into Pluronic® F127 gel had sustained drug release where ~45% of the encapsulated drug was released in 2 days. Latanoprost liposomal gels had neither irritation nor toxic effects on the rabbits' eyes. Further, they had a sustained reduction in the rabbit's intraocular pressure over a period of 3 days, which was significantly longer than that achieved by the commercial latanoprost eye drops.

S. Rathod and S. G. Deshpande[24]., designed and evaluated prolonged release drug delivery system of pilocarpine nitrate was made by optimizing thin layer film hydration method. Egg phosphatidylcholine and cholesterol were used to make multilamellar vesicles. Effects of charges over the vesicles were studied by incorporating dicetylphosphate and stearylamine. Various factors, which may affect the size, shape, encapsulation efficiency and release rate, were studied. Liposomes in the size range 0.2 to 1 μm were obtained by optimizing the process. Encapsulation efficiency of neutral, positive and negatively charged liposomes were found to be 32.5%, 35.4% and 34.2%, respectively. Biological response in terms of reduction in intraocular pressure was observed on rabbit eyes. Pilocarpine nitrate liposomes were lyophilized and stability studies were conducted.

Thi Lan Nguyen *et al*[25]., developed and in vitro evaluated liposomes using soy lecithin to encapsulated paclitaxel. Paclitaxel liposomes were prepared by thin film method using soy lecithin and cholesterol and then were characterized for their physiochemical properties such as particle size, polydispersity index, zeta potential, and morphology. The results indicated that paclitaxel liposomes were spherical in shape with a dynamic light scattering (DLS) particle size of 131 ± 30.5 nm. Besides, paclitaxel was efficiently encapsulated in liposomes, 94.5 ± 3.2% for drug loading efficiency, and slowly released up to 96 h, compared with free paclitaxel. More importantly, cell proliferation kit I (MTT) assay data showed that liposomes were biocompatible nanocarriers, and in addition the incorporation of paclitaxel into liposomes has been proven successful in reducing the toxicity of paclitaxel. As a result, development of liposomes using soy lecithin may offer a stable delivery system and promising properties for loading and sustained release of paclitaxel in cancer therapy.

Ravindra kamble *et al*[26]., developed and characterized liposomal drug delivery system for Nimesulide by various techniques such as ethanol evaporation and rotary evaporator method. The encapsulation of Nimesulide into liposomes significantly improves their properties. In spite of the numerous advantages of using liposomes as carriers to deliver Nimesulide over the free form of the drug, in vitro studies of liposome-encapsulated Nimesulide have been mainly focused on evaluation of better method of Nimesulide liposomes which have high drug entrapment, vesicle size and drug release. The average particle size, percent drug entrapment, drug release at the end was found to be 270-703µm, 49-58 %, and 65.71 % at 9 hours in case of ethanol injection method while in case of rotary evaporator it was found to be 1-12µm, 69-86% and 76.97% at 9 hours respectively. The Zeta potential for Nimesulide loaded liposomes of ethanol injection method (batch- 1) and rotary evaporator method (batch – 3) were -21.23 and -26.78 mV respectively. The result obtained in this study rotary evaporator technique was better for Nimesulide liposomes preparation on the basis of stability, drug entrapment efficiency and ethanol injection method was better on the basis of small size of liposomes and sustains release of drug when compared to rotary evaporator method and pure drug.

Devi R *et al*[27]., prepared and evaluated the topical liposomes of Fluconazole by thin film hydration technique using different ratios of soya phosphotidyl choline and

cholestrol. The in-vitro diffusion study was carried out by dialysis membrane using both open ended tube. The study was carried out in 40 ml of phosphate buffer solution pH 7.4. The percentage cumulative release from the optimized batch i.e. F7 with drug: lecithin: cholesterol ratio 1: 10: 5, found to be 75.02% release in 8 hours. The magnitude of drug retention within the vesicles on storage under defined conditions ultimately governs shelf life of the developed formulations. Liposomes showed an increasing vesicle size in accelerated temperature but no significance changes at 4±2°C has observed in storage studies for two months.

Bahareh Sabeti *et al*[28]., developed and characterized liposomal doxorubicin hydrochloride with palm oil by freeze thaw method. Their study focuses on the utilization of palm oil in formulating liposomal doxorubicin for minimizes toxicity and enhances target delivery actions by replacing phosphatidylcholine with 5% and 10% palm oil content. Liposomes were formed using the freeze thaw method, and Doxorubicin was loaded through pH gradient technique and characterized through in vitro and ex vivo terms. Based on TEM images, large lamellar vesicles (LUV) were formed, with sizes of 438 and 453 nm, having polydispersity index of 0.21 ± 0.8 and 0.22 ± 1.3 and zeta potentials of about −31 and −32 mV, respectively. In both formulations, the entrapment efficiency was about 99%, and whole Doxorubicin was released through 96 hours in PBS (pH = 7.4) at 37∘ C. Comparing cytotoxicity and cellular uptake of LUV with CaelyxR on MCF7 and MDA-MBA 231 breast cancer cell lines indicated suitable uptake and lower IC50 of the prepared liposomes.

Ehab I. Taha *et al*[29]., designed and evaluated liposomal colloidal systems for ocular delivery of ciprofloxacin. The aim of their study was enhance occular drug delivery for protective mechanism of eye is limited the bioavailability of drug. In this study several liposomal formulations containing ciprofloxacin have been formulated using reverse phase evaporation technique with final dispersion of pH 7.4. Different types of phospholipids including Phosphatidylcholine, Dipalmitoyl phosphatidylcholine and Dimyristoyl-sn-glycero-3-phosphocholine were utilized. The effect of formulation factors such as type of phospholipid, cholesterol content, incorporation of positively charging inducing agents and ultrasonication on the properties of the liposomal vesicles was studied. Bioavailability of selected liposomal formulations in rabbit eye aqueous humor has been investigated and compared with that

of commercially available ciprofloxacin eye drops (Ciprocin). Pharmacokinetic parameters including Cmax, Tmax, elimination rate constant, t1/2, MRT and AUC0–1, were determined. The investigated formulations showed more than three folds of improvement in ciprofloxacin ocular bioavailability compared with the commercial product.

**Eskandar Moghimipour *et al*[30]., ** formulated and evaluated topical liposomal gel of triamcinolone acetonide. Liposomes containing triamcinolone acetonide were prepared using thin film method. The aim of their study was to formulate and evaluate liposomal vesicles loaded with triamcinolone acetonide. The quantities of lecithin and cholesterol were changed to enhance the encapsulation of the drug. Carbomer 940 was used as gel base and four different gel formulations including hydroalcoholic gel, MLV liposomal gel, SUV liposomal gel and blank MLV gel containing free drug were prepared. The release profile of triamcinolone acetonide was determined using dialysis membrane method. Liposomes were also characterized by optical microscope and their particle size was also determined. Formulation containing lecithin: drug: cholesterol (100: 10: 10) having about 90.05% encapsulation was selected as the best formulation and the results of release showed SUV liposomal gel has the most regular and the least interaction between the drug and polymer. Results of particle size determination showed 50% of MLV and SUV liposomes had diameter below 33.80 μm and 22.09 μm, respectively. The results of characterization of the vesicles indicated the potential application of triamcinolone acetonide loaded liposome as carrier system.

B. R. Srinivas Murthy *et al*[31]., ** formulated and evaluated liposomes loaded with mupirocin. Their study aimed at developing and optimising liposomal formulation of Mupirocin, a broad spectrum antibiotic of maximum therapeutic efficacy with minimal side effects by lipid film hydration technique using various ratios of soya lecithin and cholesterol. Upon pre-formulation studies and optimization, the various formulations (of varying proportions) were prepared and subjected for various physico-chemical evaluation studies i.e., morphology, particle size, drug entrapment efficiency, *in-vitro* drug release, release kinetics and stability studies. Among five formulations (F1- F5) F4 formulation emerged as the most satisfactory formulation in all the evaluation parameters. F4 showed a maximum drug entrapment of 71.72%, average particle size was 18.3 μm, maximum percentage yield 89.06%.** The liposomes were found to be

stable during their stability studies when stored at different temperatures. They concluded that Mupirocin can also be loaded in liposomal carriers which found to be effective, stable.

Behzad Sharif Makhmalzadeh *et al*[32]., prepared and evaluated mafenide acetate liposomal formulation as eschar delivery system. Liposome formulations were prepared by two different methods such as Solvent evaporation method and microencapsulation vesicle (MCV) method. The prepared liposomes undergoes experimental design and data analysis. Drug/lipid ratio, hydration time, aqueous phase volume and homogenizer rpm were considered as independent variable, on the other hand, liposome size, drug loading, stability, drug release and skin permeability parameters as responses. The results demonstrate that liposome were multilamellar and multivesicular. Particle size and drug loading percentage of MCV liposome indicated burst sustained release profile. Burst effect in solvent evaporation liposome was more than MCV liposome. In their conclusion, solvent evaporation liposome improved mafenide acetate partitioning through rat skin and decrease diffusion coefficient with increase particle size of liposome.

Srinivas Lankalapalli *et al*[33]., prepared and evaluated liposome formulations for poorly soluble drug Itraconazole by complexation. Beta cyclodextrin and Hydroxy propyl beta cyclodextrin inclusion complexes with Itraconazole were prepared by kneading method/ solvent injection method and these complexes were incorporated in the aqueous phase of the liposomes to prepare Itraconazole liposomes. Factor such as ratio of lipids employed, drug:lipid ratio, etc were fine tuned and optimized to achieve maximum entrapment of the Itraconazole in the aqueous phase. The prepared liposomes are characterized by optical microscopy, scanning electron microscopy, particle size determination, encapsulation efficiency and also evaluated by using FTIR spectroscopy and in-vitro diffusion studies by using dialysis membrane. . The drug content was in the range of 94.78 % w/w to 101.81 % w/w for the liposome formulations. The encapsulation efficiency was found to be 37.99 % to 55.01 %. The percentage drug release was found to be 17.25% to 39.62%. The increase in the solubility of Itraconazole with cyclodextrin complexes in comparision with plain drug is an indubitable advantage of this approach.

Anayatollah Salami *et al*[34]., formulated and evaluated liposomes for transdermal delivery of Celecoxib. Liposomes were prepared by thin film method using soya lecithin and cholesterol. Physicochemical characteristics of the liposomes such as, particle size, drug encapsulation efficiency, drug release and in vitro skin permeability through rat skin were evaluated using Franz diffusion cells were determined. The results showed that the maximum drug encapsulation efficiency was 43.24%. Drug release profile showed that 81.25% of the drugs released in the first 24 hours of the experiment. The decrease of lecithin increased values. Particle sizes of the formulations ranged from 0.117 to 1.123 µm. Jss, Dapp and P parameters in L - 8 formulations were 29.18, 60.95, and 3.21 times higher than those of saturated water solution of celecoxib, respectively. The results of vesicles characterization indicated the potential application of celecoxib loaded liposome as carrier system. In conclusion, the components such as lecithin and cholesterol, and vortex time in liposomal formulations have an essential role in the physicochemical properties and celecoxib permeability through rat skin.

U. D. Shivhare *et al*[35]., formulated and evaluated liposome formulation of pentoxifylline. Liposomes were prepared by physical dispersion method using different ratio of lipids. In evaluation study, the effect of the varying composition of lipids on the properties such as encapsulation efficiency, particle size and drug release were studied. Phase transition study was carried out to confirm the complete interaction of pentoxifylline with bilayer structure of liposome. Moreover, the release of the drug was also modified and extended over a period of 8 h in all formulations. The average particle size, percent drug entrapment, drug release at the end was found to be 6.24 - 15.07 µm, 29.64 - 48.92 %, and 90.0- 99.23 % at 8 hours. In concluison, release of the drug from the most satisfactory formulation was evaluated through dialysis membrane to get the idea of drug release.

Yan Chen *et al*[36]., Prepared Curcumin-Loaded Liposomes and evaluated their skin permeation and pharmacodynamics. Liposomes were prepared by the conventional film method. Soybean phospholipids (SPC), egg yolk phospholipids (EPC), and hydrogenated soybean phospholipids (HSPC) were selected for the preparation of different kinds of phospholipids composed of curcumin-loaded liposomes: C-SPC-L (curcumin-loaded SPC liposomes), C-EPC-L (curcumin-loaded EPC liposomes), and C-HSPC-L (curcumin-loaded HSPC liposomes). The physical properties of different

lipsomes were investigated as follows: photon correlation spectroscopy revealed that the average particle sizes of the three types of curcumin-loaded liposomes were 82.37 ± 2.19 nm (C-SPC-L), 83.13 ± 4.89 nm (C-EPC-L), and 92.42 ± 4.56 nm (C-HSPC-L), respectively. The encapsulation efficiency values were found to be 82.32 ± 3.91%, 81.59 ± 2.38%, and 80.77 ± 4.12%, respectively. An in vitro skin penetration study indicated that C-SPC-L most significantly promoted drug permeation and deposition followed by C-EPC-L, C-HSPC-L, and curcumin solution. Moreover, C-SPC-L displayed the greatest ability of all loaded liposomes to inhibit the growth of B16BL6 melanoma cells. Therefore, the C-SPC-L were chosen for further pharmacodynamic evaluation. A significant effect on antimelanoma activity was observed with C-SPC-L, as compared to treatment with curcumin solution in vivo. Their results suggest that C-SPC-L would be a promising transdermal carrier for curcumin in cancer treatment.

Xiang-qi QIN *et al*[37]., prepared a novel transdermal preparation of liposomal brucine (LB) and investigate its pharmaceutical/pharmacodynamic characterization. LB was prepared by a modified ethanol-dripping method. Its drug encapsulation efficiency (EE), particle size, in vitro release, and skin permeation were studied. Furthermore, a safety evaluation and pharmacodynamic analysis of LB, including acute dermal toxicity, skin irritation, and analgesic and anti-inflammatory effects were investigated. The EE of LB was 72% and the mean particle size of the liposomes was 55.4 nm. The in vitro release profile indicated that less than 68% of the encapsulated brucine was released in 10 h. A skin permeation study showed that compared with the free brucine, LB exhibited higher cumulative drug permeation through the skin and lower drug accumulation in skin tissue, indicative of an obvious promotion of skin permeation with liposomal encapsulation. The acute dermal LD50 of LB was greater than 100 mg/kg (brucine content) and skin irritation tests revealed that LB had no irritation to both integrity and broken skin. A pharmacodynamic evaluation of LB was performed by xylene-induced mouse ear edema test and acetic acid-induced writhing test at the dosage of 1.5, 3, and 6 mg/kg, respectively. The results showed that anti-inflammatory activities and analgesic effects of brucine encapsulated were significantly higher than that of the free brucine ($P<0.01$). It can be proposed that LB prepared here could represent a safe, effective and promising transdermal formulation for analgesic and anti-inflammatory effects.

P.Divakar *et al*[38]., formulated and in vitro evaluated liposomes containing metformin hydrochloride. Liposomal suspensions were prepared using film hydration technique using varying concentrations of phosphatidylcholine and cholesterol and optimize the ideal combination for required drug release. Liposomal formulations were evaluated for particle size, drug entrapment and in-vitro drug release studies. The percentage drug release at the end was found to be 64.0- 83.0 % at 4 hours. Drug excipient compatibility was determined by using U.V spectroscopy, FT-IR spectral studies. The results of in vitro drug release studies showed that release from liposomal formulation was slow and sustained for >12 hrs period. The formulations followed first order kinetics and release mechanism was non-fickian diffusion from all the formulations.

Satyavathi K *et al*[39]., formulated and in-vitro evaluated liposomal drug delivery system of cabazitaxel. Cabazitaxel liposomes were prepared by thin film hydration technique using lecithin, cholesterol, and Tween 80. Six formulations of liposomes were evaluated for physico chemical properties and in vitro drug release. The compatibility of drug with other ingredients was checked by FTIR studies. The prepared liposomes were characterized for surface morphology by SEM analysis, Percentage drug entrapment efficiency, Particle size, and Zeta potential analysis. The average particle size, poly dispersive index, percentage drug entrapment efficiency and zeta potential analysis as found to be 317 nm to 564 nm, 0.632 to 0.762, 78.39% to 82.96% and -15.2mV to -20.9mV .The in-vitro drug release for optimized formulation F2 followed zero-order release kinetics. F2 was formulated using 270 mg lecithin and 30 mg cholesterol and 0.5 ml of Tween 80 with an objective to achieve a linear release profile for 24 hr. There was no initial burst release, with 5.68% of drug released during the first hour and release was extended up to 24 hrs. Stability studies at different temperatures were conducted and maximum drug retention was found at refrigerated temperature 4ºC.

Anjana ashok adhyapak, and Babasaheb gangadhar desai[40]., formulated and evaluated liposomal transdermal patch for targeted drug delivery of tamoxifen citrate for breast cancer. Liposomes were formulated by solvent evaporation method using poly (sebacic acid-co-ricinoleic acid) in varying ratios and evaluated for particle size, drug loading, entrapment efficiency, transmission electron microscopy,

differential scanning calorimetry, and X-ray diffraction. Formulated tamoxifen-loaded liposomes were finally incorporated into transdermal patch and evaluated for thickness drug content, moisture content, moisture uptake, folding endurance, tensile strength diffusion coefficient, permeability coefficient, in vitro permeation, and skin irritation. Optimized transdermal patches were tested for its pharmacokinetic and pharmacodynamics parameters. The average particle size, percentage drug entrapment efficiency and percentage drug release was found to be 412-543nm, 90.32 -93.33%. It can be concluded Optimized patch formulation F3 was found to have the best release profile when compared to other patches with no skin irritation. Pharmacokinetic and pharmacodynamics profile of formulated patch confirms its advantage over other conventional existing formulations.

Shruthi M V *et al*[41], formulated and evaluated proliposomal gel containing metformin hydrochloride using mannitol as a water soluble carrier. Proliposomes of Metformin hydrochloride were prepared by thin film hydration technique by varying the composition drug, manitol, soya lecithin and cholesterol. Proliposome formulations were characterized for compatibility, vesicle size, % Drug content, % Entrapment efficiency, Surface morphology, Surface charge, invitro drug release and stability studies. The proliposomal gel was prepared for optimized proliposomal formulation F4 by incorporated into 1% Carbopol gel. The in vitro drug release and in vivo skin irritation study and hypoglycemic activity were carried out for the gel F4. Drug and physical mixture were characterized by FTIR, the result of IR study showed that no interaction between drug and polymers and other formulation parameters of formulated proliposomes and proliposomal gel are evaluated which showed better results. Proliposomal gel F4 was proved nonirritant and showed better stability, more hypoglycemic effect as compared to oral formulation because it provide reduction in blood glucose level with controlled manner upto 24 hrs. Hence, Proliposomes drug delivery system was better choice for sustained release of drug through topical drug delivery.

Sangeetha S. S. and Roopa Karki[42]., formulated and evaluated liposomes in carbopol gels for mixed vaginal infections. The combination of voriconazole and metronidazole were selected as model drugs for mixed vaginal infections. Multi lamellar liposomes composed phosphatidylcholine and cholesterol along with

combination of drugs was prepared by the thin film hydration method. The prepared liposome were characterized for FT-IR, size distribution, entrapment efficiency, in vitro release study in simulated vaginal fluid and stability studies. The liposomes were loaded to carbopol gel. The liposomes loaded carbopol gels were evaluated for in vitro drugs release study and compared with control gel. FTIR study indicated that there is no significant chemical interaction between the components. The cumulative percent release from liposomal gels FL1 was found to be 69.90% for metronidazole and 56.02 % for voriconazole. In vitro release studies of liposomes incorporated in the carbopol gel have shown a prolonged release of entrapped metronidazole and voriconazole compared to control gel. Stability studies showed that the vesicles were stable in refrigerated temperature (4°C) for 60 days without significant differences in drug entrapment.

Krishna Mohan Chinnala and Rabinarayan Panigrahy[43]., formulated and evaluated of acyclovir liposomes. The liposomes were prepared by rotary flash evaporation method and thin film hydration technique using different ratios of phospholipids and cholesterol with a desired amount of drug. The percentage entrapment efficiency was determined and maximum entrapment efficiency was found to be 99%. The optimized batches were found to have good entrapment efficiency and it was proved that as the concentration of the cholesterol increased the particle size also increased and entrapment efficiency decreased. In formulation TB1 the ratio of cholesterol and lecithin is 1:1 is selected as optimized formulation based on entrapment efficient and diffusion studies Optimized formulation was evaluated for particle size analysis and zeta potential. FTIR studies were done on the pure drug and physical mixture of drug and polymers. From FTIR spectra of the drug and physical mixture it was found that there is no significant interaction. Zeta potential analysis was done for optimized formulation TB1. Average zeta potential and charge on the liposome was determined. The value was -20 Mv which indicates that the surface of liposomes is dominated by the anions and proved that prepared liposome have sufficient charge to avoid aggregation of vesicles. It can be concluded that as the concentration of the cholesterol increased the particle size also increased and entrapment efficiency decreased.

Sunitha Sampath *et al*[44]., prepared and evaluated liposome entrapped hydrogel complex systems of itraconazole for enhanced transdermal permeation. The liposomes were prepared by thin flim hydration method. The complex systems were prepared by incorporating drug liposome consisting of biocompatible lipid, into carbopol to form hydrogel. The systems were evaluated for encapsulation efficiency, particle size, zeta potential and ex vivo release behavior for skin permeability. FT-IR studies were done to find for any drug excipient interactions. The particle size was ranging from 94.2 nm to 104.8 nm with low PdI indicating the formation of monodisperse system. The % of drug released from the formulation was ranging from 48.04 % to 99.92 % in 24 h. In terms of skin permeability, complex liposomal hydrogel has proved to have greater skin permeation compared to simple liposomal system, simple hydrogel system and the plain drug suspension (485.49, 362.06, 226.03 and 172.25 μg/cm^2 /h1). From the release kinetics concluded the drug is releasing by diffusion mechanism and also due to erosion of the gelling agent. It was found that liposome in hydrogel complex systems improved skin permeability of the drug when compared to control with high flux and high permeability coefficient. These results indicate that liposome in hydrogel systems can function as probable drug delivery systems to enhance transdermal permeation of the water insoluble itraconazole for treating the topical infections.

Dr. Khaja Pasha and Dr. Shahana Banu[45]., formulated and evaluated glimepiride liposomal drug delivery system. Liposomes were prepared by physical dispersion method using different ratio of lipids. In evaluation study the effect of the varying composition of lipids on the properties such as encapsulation efficiency, particle size and drug release were studied. Phase transition study was carried out to confirm the complete interaction of Glimepiride with bilayer structure of liposome. Moreover, the release of the drug was also modified and extended over a period of 8 h in all formulations. F1 emerged as the most satisfactory formulation in so far as its properties were concerned. Further, release of the drug from the most satisfactory formulation (F1) was evaluated through dialysis membrane to get the idea of drug release.

Akshay Singha Roy *et al*[46]., designed, formulated and evaluated liposome containing isoniazid. Liposome of isoniazid was made by thin layer film hydration method. Six batches of liposomes were prepared based on the different weight ratio of

L-α-phosphatidylcholine and cholesterol. Differential scanning calorimetry (DSC) study conducted to study in any incompatibility. The prepared liposomes were evaluated by particle size analysis, entrapment efficiency, release study and stability study. Particle sizes were determined from the scanning electron microscopy (SEM) photographs. When particle frequencies were plotted against particle diameter in the histogram, it showed that F1 batch had a skewed distribution towards smaller liposomes while F6 shows a proper bell-shaped curve with a mean at 225 μm. The percentage entrapment efficiency was found to be 4.19 ± 0.12 to 8.99 ± 0.15% . From the release profile, it was seen that F1 batch was fastest and F6 was slowest to release the drug. The satisfactory batch F1 was packed in Eppendorf tube and stored at 4°C temperature for one month. At the end of one month, the samples were analyzed for their physical properties, drug entrapment and in vitro release profile. The percentage release was found to be 96.5 ± 3.2 after 4 hr. The F1 batch showed most promising results compared to other. No significant change was found during one month's stability study of final batch (F1).

M. P. Jadhav *et al*[47]., formulated and evaluated long circulating liposomes for amphotericin B. Liposomes were prepared by simple film hydration method. The formulation was optimized using 2^3 factorial designs. Pegylated liposomal formulation showed favorable results with reference to particle size (247.33±9.60 nm), percent entrapment efficiency (94.55±3.34%). TEM studies revealed that the liposomes were essentially spherical, hollow, and appeared like powder puff structures. From DSC study it was concluded that the pegylated formulation containing Amp B showed better stability and membrane integrity of the formulation. During the stability studies the formulation was found to be stable. When subjected to gamma scintigraphy kinetic tracer studies the formulation showed longer residence time in the blood in BALB/C mice.

Toshimasa Tsukamoto *et al*[48], prepared bromfenac-loaded liposomes modified with chitosan for ophthalmic drug delivery and evaluated physicochemical properties and drug release profile. Bromfenac (BRF)-loaded liposomes were prepared using the calcium acetate gradient method. Liposome sizes and encapsulation efficiencies were optimized by screening several liposome formulations of lipid, drug concentration, and buffer solution. BRF entrapment efficiency was greater than 90% using this method,

and was low using conventional hydration methods. High initial BRF loading using the pH gradient method caused aggregation of liposomes. To circumvent aggregation, the negatively charged lipid dicetylphosphate was incorporated into liposomes, which formed anion layer preventing coalescence. Release of BRF from liposomes was sustained for several hours depending on lipid concentration, inner water phase, initial drug amounts, and surface properties. Surface modification with chitosan (CS), a mucoadhesive cationic polymer, was achieved using electrostatic interactions of negatively charged liposomes. The optimal concentration of CS for evasion of liposome aggregation was 0.15%. The model NSAID BRF was encapsulated into liposomes using the pH gradient method. Formulations were optimized to achieve almost 100% drug loading efficiency.

Dr. M. Purushothaman *et al*[49], prepared and evaluated decitabine liposmoes. Decitabine Liposomes were prepared by the thin film hydration method using the soya lecithin as the phospholipid. Their study mainly explains about the effect of concentration of soya lecithin, cholesterol and Tween 80. The prepared liposomes were characterized by scanning electron microscopic method respectively. The In-vitro release studies were performed and the drug release kinetics was evaluated using linear regression method. The objective of the present study was to develop liposome containing Decitabine and the prepared liposomes were evaluated for size, shape, drug entrapment efficiency, In-vitro drug release and stability. Decetabine loaded liposomes formulation had good ability to encapsulate drug and elicted favorable physicochemical characteristics. The intestinal absorption and antitumor capacity of Decitabine was significantly enhanced by using liposomes. These results suggest that liposomes could be a promising perioral carrier for Decitabine.

Deevan Paul And Vothani Sarath Babu[50]., formulated and evaluated liposomal gel containing ketoconazole. Liposomes were prepared by thin film hydration technique using ketoconazole, Soya lecithin, Cholesterol were dissolved in mixture of chloroform and methanol (9:1) were taken in different levels. 0. The FT-IR spectroscopy study was carried out to check out the compatibility between the drug Ketoconazole and the excipients used for the preparation. These include average particle size and size distribution, shape, encapsulation efficiency, Percentage drug content, In-vitro drug release study. The compatibility study of the prepared

Ketoconazole Liposomes showed no interaction between drug and excipients. The size of liposomal formulations ranged from 3.278- 19.688µm. The entrapment efficiency of drug increased when molar ratio of lipid to cholesterol was changed from 1:1% Drug content of liposomal formulations obtained shows 94.6 - 99.2% drug content. Liposomal dispersion and gel were found to increase the skin permeation and deposition compared to control and marketed gel. They concluded that Ketoconazole can also be loaded in liposomal carriers which found to be effective, stable and can be preceded for further future studies.

Mohamed A. El-Nabarawi *et al*[51]., formulated and evaluated dispersed paroxetine liposomes in gel. Paroxetine liposomes were prepared by reverse phase evaporation technique using soya lecithin, cholesterol and drug in different weight ratios. The prepared liposomes were characterized for size, shape, entrapment efficiency. The effect of using different weight ratios of soybean lecithin phosphatidylcholine: cholesterol on entrapment efficiency and on drug release was studied. Liposomes showed entrapment efficiency percent of 81.22% ± 3.0 8 for paroxetine. The optimized paroxetine liposomes formula was F5 (7:7) molar ratio of (SLP: CHOL), which after that was incorporated in different based gels at different concentrations as Pluronic F127 (PF127-G) (20%, 25%and 30%), Carbopol 934 (C934-G) (1%, 1.5% and 2%) and Hydroxypropyl methylcellulose E4M (HPMC-G) (2%, 4% and 6%) and evaluated through in-vitro release, viscosity, pH and drug content.

M.Yasmin Begum *et al*[52]., designed and evaluated flurbiprofen liposomes. Liposomes were prepared by thin film hydration technique using various lipids such as soyphosphatidyl choline, dipalmitoyl phosphatidyl choline and distearoyl posphatidyl choloine with or without cholesterol. All the prepared formulations were characterized for their physico chemical properties such as appearance, vesicle size, vesicle size distribution, percentage drug entrapment, stability of the liposomes in terms of their drug leakage and drug retention behavior. Stability was studied by storing the liposomal formulations under different conditions for the period of 30 days. The optimized formulation parameters and process parameters resulted the liposomes in the vesicle size range of 4.6±0.6 to 5.6±0.4µm with mean vesicle diameter of 5. The maximum percentage drug entrapment was achieved with the formulation which contains the distearoyl phosphatidyl choline and cholesterol. In vitro percentage drug release data

showed that the release profile follows zero order kinetics and mechanism of drug release was diffusion. The flurbiprofen liposomes with good stability and appreciable controlled drug release with good retention of the drug even after 24 hours were prepared successfully

J S Dua *et al*[53]., prepared, optimized, characterized liposomes containing serratiopeptidase for oral delivery. The liposome prepared by ethanol injection method by using Phosphatidylcholine and cholesterol at 10:10 ratio shown good % Entrapment efficiency and In-vitro drug release study. Optimized formulations were subjected to stability studies at two different temperatures for up to 45 day. No significant changes in drug entrapment efficiency and mean particle size were observed during the course of stability study for formulations stored at 4-8ºC but there was a significant decrease in drug entrapment efficiency for liposome stored at room temperature after 15, 30 and 45 days and the mean particle size were increased at room temperature after 15, 30 and 45 days.

Materials and Methods

LIST OF CHEMICALS

LIST OF CHEMICALS

Table No. 1

S. No	INGREDIENTS	SOURCE
1.	Metformin HCL	Reachem laboratory chemicals, Chennai
2.	Cholesterol	Reachem laboratory chemicals, Chennai
3.	Soy lecithin	The urban platter food co., Mumbai
4.	Chloroform	RANKEM, Haryana
5.	Ether	RANKEM, Haryana
6.	Sodium hydroxide	Reachem laboratory chemicals, Chennai
7.	Potassium di hydrogen phosphate	Merck specialities pvt. Ltd., Mumbai

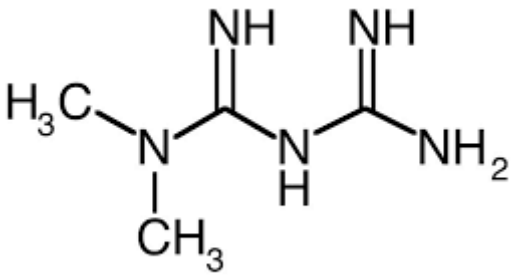
DRUG PROFILE

DRUG PROFILE[54, 55, 56]

METFORMIN HCl

DRUG NAME : Metformin hydrochloride

STRUCTURE



MOLECULAR FORMULA : $C_4H_{11}N_5$

MOLECULAR WEIGHT : 129.1636

CHEMICAL NAME : 1-carbamimidamido-N,N-dimethylmethanimidamide

CATEGORY : Anti-hyperglycemic drug

DOSE : 500 -2500mg

DESCRIPTION : white crystalline powder, odorless, bitter taste

SOLUBILITY : Freely water soluble

MELTING POINT : 223-226 °C

MECHANISM OF ACTION:

Metformin's mechanisms of action differ from other classes of oral anti-hyperglycemic agents. Metformin decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization. These effects are mediated by the initial activation by metformin of AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats. Activation of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cells. Increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors. Metformin administration also increases AMPK activity in skeletal muscle. AMPK is known to cause GLUT4 deployment to the plasma membrane, resulting in insulin-independent glucose uptake.

PHARMACOKINETICS:

Absorption:

Absorbed over 6 hours, bioavailability is 50 to 60% under fasting conditions. Administration with food decreases and delays absorption. Some evidence indicates that the level of absorption is not dose-related, suggesting that absorption occurs through a saturable process. Limited data from animal and human cell cultures indicate that absorption occurs through a passive, non-saturable process, possibly involving a paracellular route. Peak action occurs 3 hours after oral administration.

Distribution:

654 L for metformin 850 mg administered as a single dose. The volume of distribution following IV administration is 63-276 L, likely due to less binding in the GI tract and/or different methods used to determine volume of distribution.

Metabolism:

Metformin is not metabolized.

Elimination:

Intravenous single-dose studies in normal subjects demonstrate that metformin is excreted unchanged in the urine and does not undergo hepatic metabolism (no metabolites have been identified in humans) nor biliary excretion. Approximately 90% of the drug is eliminated in 24 hours in those with healthy renal function. Renal clearance of metformin is approximately 3.5 times that of creatinine clearance, indicating the tubular secretion is the primary mode of metformin elimination.

ADVERSE REACTION:

Lactic acidosis is characterized by elevated blood lactate levels (>5mmol/L), decreased blood pH, electrolytic disturbance with an increased anion gap and an increased lactate / pyruvate ratio. Feeling very weak, tired or unusual muscles pain, breathing trouble, unusual stomach discomfort were observed with Metformin. Metformin hydrochloride therapy may produce unpleasant or metallic taste, feeling cold, dizziness and suddenly developing a slow or irregular heartbeat. Gastrointestinal symptoms like diarrhea, nausea, vomiting, abdominal bloating, flatulence and anorexia are the most common but very rare (approximately 3%) symptoms.

INDICATIONS:

Metformin is used in patients with type 2 diabetes (non - insulin dependent diabetes). Controlling high blood sugar helps prevent kidney damage, blindness, nerve problem, loss of limbs and sexual function problem. Proper control of diabetes may also lessen the risk of a heart attack or stroke. It also decreases the amount of sugar that the liver makes and that the stomach /intestines absorb.

DOSAGE AND ADMINISTRATION:

The usual starting dose of Metformin hydrochloride 500mg tablet is, one tablet twice in a day, given with the morning and evening meals. Metformin hydrochloride can be administered up to 2000mg per day (twice a day). The maximum recommended daily dose for Metformin hydrochloride in adult is 2000mg.

CONTRAINDICATION:

Metformin is contraindicated in people with any condition that could increase the risk of lactic acidosis, including kidney disorders, lung disease and liver disease. Metformin is recommended to be temporarily discontinued before any radiographic study involving iodinated contrast agents (such as contrast – enhanced CT scan or angiogram), as the contrast dye may temporarily impair kidney function, indirectly leading to lactic acidosis by causing retention of Metformin in the body.

MARKETED PRODUCTS:

Metlong – D, Glumet, Sumet, Daomet, Diamet, Gluconorm, GluforminXL, K-met, Glyciphage, Melmet, Obimet, Zoform.

OTHER DOSAGE FORMS:

Pio – MF bilayered tablets, Glycheck – MF conventional tablets, Riomet oral solution.

EXCIPIENT PROFILE

SOYA LECITHIN[57, 58, 59, 60]

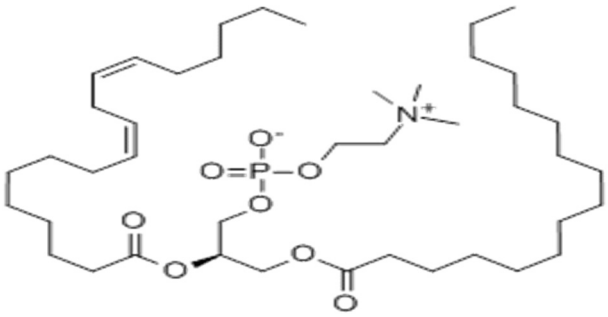
NON – PROPRIETARY NAME : Lecithin

SYNONYMS : Mixed soybean phosphatides, ovolecithin, mixed soybean phosphatides, ovolecithin, soybean lecithin, soybean phospholipids, vegetables lecithin.

EMPRICAL FORMULA : $C_{42}H_{80}NO_8P$

MOLECULAR WEIGHT : 758.06g/mol

STRUCTURAL FORMULA :



MELTING POINT : -5˚C (23˚F)

FUNCTIONAL CATEGORY : Emollient; emulsifying agent; solubilizing agent.

DESCRIPTIONS : Solid, White or faintly yellow pearly granules or crystals

SOLUBILITY : Insoluble in water, soluble in chloroform, ethanol and ether.

STABILITY	**:**	Stable, but light, heat, moisture and air-sensitive. Incompatible with strong oxidizing agents.
APPLICATIONS	**:**	Hypolipidemic ingredients , nutrition supplements, humectant and emulsifiers
STORAGE	**:**	Do not store in direct sunlight. Store in a tightly closed container. Store in a cool, dry, well-ventilated area away from incompatible substances. Storage temperatures should not exceed 75ºF.
STABILITY	**:**	Stable under ordinary conditions of use and storage.
INCOMPATIBILITIES	**:**	Excessive heat and flames, Avoid strong oxidizers, incompatible with esterases owing to hydrolysis.

CHOLESTEROL[61, 62]

NON- PROPRIETARY NAME : BP: Cholesterol,

JP: Cholesterol,

PhEur: Cholesterol,

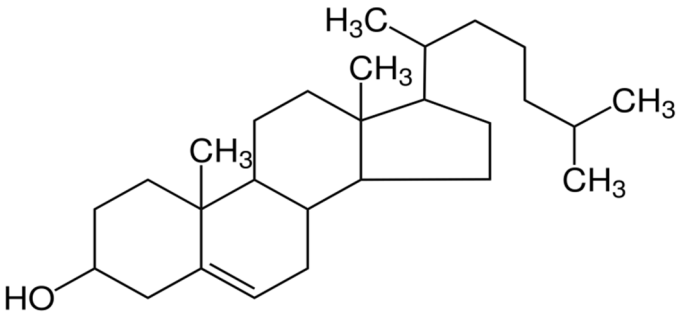
USP-NF: Cholesterol

SYNONYMS : Cholesterin, cholesterolum.

EMPRICAL FORMULA : $C_{27}H_{46}O$

MOLECULAR WEIGHT : 386.664 g/mol

STRUCTURAL FORMULA :



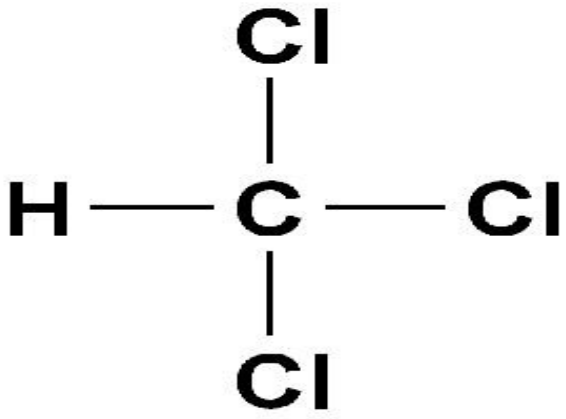
MELTING POINT : 148.5 °C

FUNCTIONAL CATEGORY : Emulsifying agent,

DESCRIPTIONS : Solid, White or faintly yellow pearly granules or crystals and white crystalline powder.

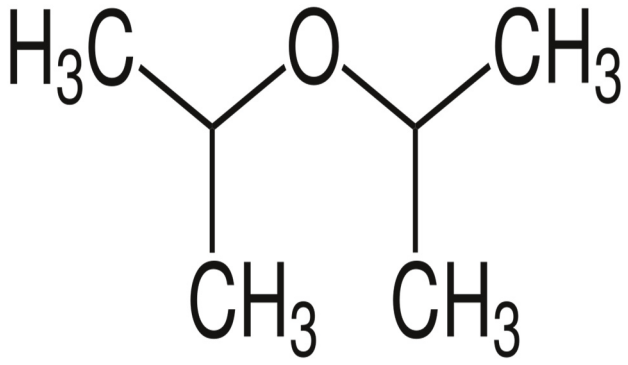
SOLUBILITY	**:**	Lower solubility in water, soluble in ethanol, methanol, hexane, acetone, ether, chloroform and benzene.
APPLICATIONS	**:**	Cholesterol is used in liposomes to encapsulate and deliver chemotherapeutic drugs to diseased tissues, Cholesterol-C14 is used clinically as an organ imaging agent. Organs visualized by the technique include ovaries, adrenals, and spleen.
STORAGE	**:**	Stored in well close container, protect from direct sunlight and away from oxidizing agent.
STABILITY	**:**	Stable under ordinary condition
INCOMPATIBILITIES	**:**	Incompatible with strong oxidizing agents

CHLOROFORM[63, 64]

SYNONYMS	:	Trichloromethane, Formyl trichloride, Trichloroform, Methenyl chloride, Methenyl trichloride.
EMPRICAL FORMULA	:	$CHCl_3$
MOLECULAR WEIGHT	:	119.369 g/mol
STRUCTURAL FORMULA	:	
MELTING POINT	:	-63.2 °C
FUNCTIONAL CATEGORY	:	Inhaled anesthetic and used as a solvent
DESCRIPTIONS	:	Clear colorless liquid with a characteristic odor
SOLUBILITY	:	Slightly soluble in water and soluble in carbon disulfide,
APPLICATIONS	:	Chloroform was used in the past as an extraction solvent for fats, oils, greases, and other products; as a dry cleaning spot remover; in fire extinguishers; as a fumigant and as an anesthetic.
STORAGE	:	It is to be stored in a cool, dry, well-ventilated place, out of direct light.

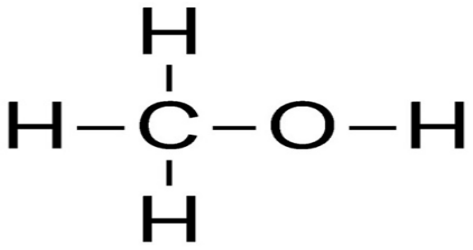
STABILITY	**:**	Decomposes at ordinary temp in sunlight in the absence of air, and in the dark in the presence of air.
INCOMPATIBILITIES	**:**	It is incompatible with dinitrogen tetraoxide, fluorine, sodium metal and alcohols, nitromethane, and triisopropylphosphine.

ETHER[65, 66, 67]

SYNONYMS	:	Diethyl ether, Ethyl ether, Ethoxyethane, Diethyl oxide, Ethyl oxide
EMPRICAL FORMULA	:	$C_4H_{10}O$
MOLECULAR WEIGHT	:	74.123 g/mol
STRUCTURAL FORMULA	:	
MELTING POINT	:	-116°C
FUNCTIONAL CATEGORY	:	Fuels and fuel additives, Functional fluids (closed systems), Propellants and blowing agents
DESCRIPTIONS	:	Clear colorless liquid with an anesthetic odor
SOLUBILITY	:	Soluble in acetone, naphtha, benzene and oils, very soluble in ethanol

APPLICATIONS	**:**	Anesthetic, It is a great solvent for fats, waxes, oils, perfumes, alkaloids, and gums.
STORAGE	**:**	Ethers should be stored in tightly closed containers within properly labelled fire resistant metal cabinets, or bins, and on drip trays. They must be stored separate from oxidizers.
STABILITY	**:**	Ether is stable under normal ambient and anticipated storage and handling conditions of temperature and pressure.
INCOMPATIBILITIES	**:**	It is incompatible with acids, bases, oxidizers, and poisons.

METHANOL[68, 69, 70]

SYNONYMS	:	Methyl alcohol, wood alcohol, carbinol, wood spirit, wood naphtha, methylol, methyl hydroxide, pyroxylic spirit, colonial spirit, columbian spirit, monohydroxymethane, methylalkohol
EMPRICAL FORMULA	:	CH_3OH
MOLECULAR WEIGHT	:	32.042 g/mol
STRUCTURAL FORMULA	:	
MELTING POINT	:	-97.6 °C
FUNCTIONAL CATEGORY	:	Industrial solvent
DESCRIPTIONS	:	Colourless liquid with characteristic odour.
SOLUBILITY	:	Miscible with ethanol, ether, benzene, most organic solvents and ketones, Soluble in acetone, chloroform
APPLICATIONS	:	Methanol is primarily used as an industrial solvent for inks, resins, adhesives, and dyes. It is also used as a solvent in the manufacture of

		cholesterol, streptomycin, vitamins, hormones, and other pharmaceuticals.
STORAGE	**:**	Store in a segregated and approved area. Keep container in a cool, well-ventilated area. Keep container tightly closed and sealed until ready for use. Avoid all possible sources of ignition (spark or flame).
STABILITY	**:**	Stored in well close container, protect from direct sunlight and away from oxidizing agent.
INCOMPATIBILITIES	**:**	It is incompatible with acids, bases, oxidizers and poisons.

LIST OF EQUIPMENTS

LIST OF EQUIPMENTS

Table No. 2

S.No	EQUIPMENT NAME	EQUIPMENT MANUFACTURER
1.	Magnetic stirrer	Remi equipment Pvt.Ltd
2.	Digital Balance	ORSON
3.	Optical microscopy	Olympus opto systems India Pvt. Ltd, Noida
4.	UV spectrophotometer	UV – 1800 SHIMADZU corporation, Japan
5.	Cooling centrifugator	Remi elektrotecnik limited, vasai, India.

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METHODOLOGY

METHODOLOGY

CONSTRUCTION OF STANDARD CURVE OF METORMIN HCL

Preparation of 0.2M Potassium di hydrogen phosphate:

Accurately weighed 27.218 gm of potassium di hydrogen phosphate was dissolved in distilled water and make up to 1000 ml with distilled water.

Preparation of 0.2M Sodium hydroxide solution:

Accurately weighed 8.0 gm of sodium hydroxide was dissolved in distilled water and make up to 1000 ml with distilled water.

Preparation of ph 6.8 phosphate buffer

500 ml of 0.2 M potassium di hydrogen phosphate and 224 ml of 0.2 M sodium hydroxide solution was mixed together and made up to 2000 ml with distilled water. Then it was adjusted to pH 6.8[71].

Preparation of standard curve of Metformin HCl using pH 6.8 phosphate buffer[72]:

Accurately weighed 100 mg metformin HCl was dissolved in water and the volume was make up to 100 ml using distilled water in a volumetric flask to obtain a solution of 1000 µg/ml. From the above solution 10 ml was pipetted out into a 100 ml volumetric flask and made up to 100 ml using phosphate buffer pH 6.8 to get a stock solution of 100 µg/ml. From this stock solution, aliquots of 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.0ml, 1.2ml, 1.4ml, 1.6ml, 1.8 ml and 2.0ml were pipetted out into a series of 10 ml volumetric flask and made up to mark with phosphate buffer pH 6.8 to get a concentration in the range of 2 to 20 µg/ml. The absorbance of the resulting solution was then measured at 233 nm using UV Double beam spectrophotometer against phosphate buffer pH 6.8 as blank. The standard curve was obtained by plotting concentration (µg/ml) values in X- axis and absorbance values in Y – axis.

STANDARD CURVE DATA OF METFORMIN HCL USING PHOSPHATE BUFFER pH 6.8

Table No. 3 Standard curve data of Metformin HCl using phosphate buffer p H 6.8

S. No.	Concentration (µg/ml)	Absorbance at 233 nm
1.	2	0.189
2.	4	0.370
3.	6	0.524
4.	8	0.699
5.	10	0.858
6.	12	1.055
7.	14	1.244
8.	16	1.394
9.	18	1.568
10.	20	1.716

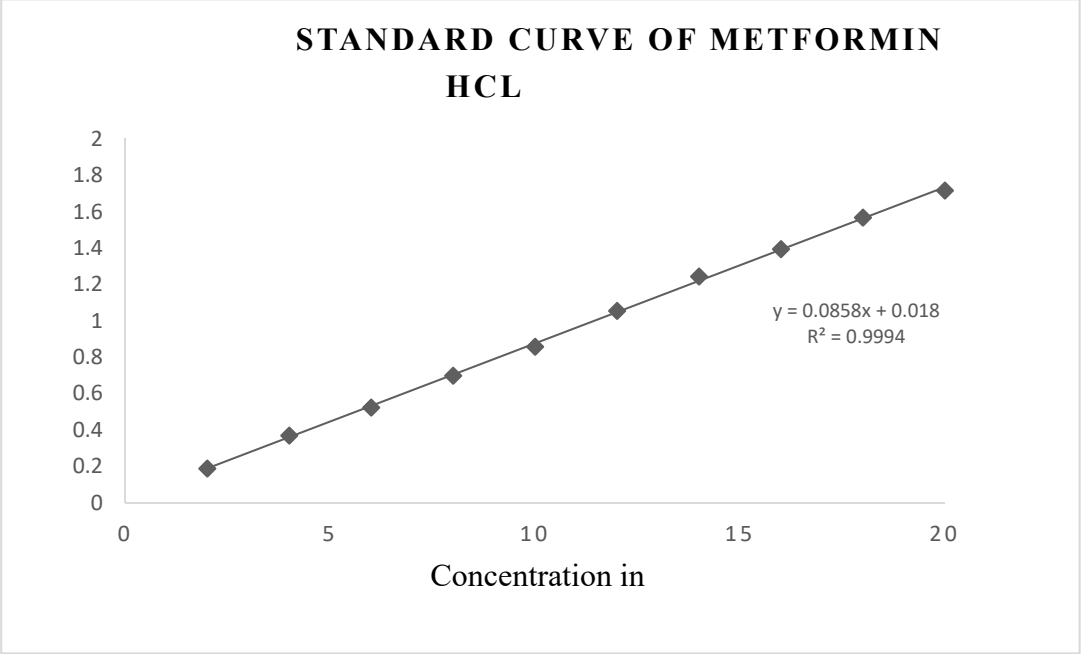


Figure No. 4 Standard curve of Metformin HCl

PREFORMULATION STUDIES

Preformulation testing is an investigation of physical and chemical properties of a drug substance alone and when combined with excipients. It is the first step in the rational development of dosage form. The objective of preformulation testing is to generate information useful to the formulation in developing stable and stable and bioavailable dosage forms. The use of preformulation parameters maximize the chances in formulating an acceptable, safe, efficacious and stable product[73].

A) Solubility

Solubility of Metformin Hcl in water, methanol, phosphate buffer pH 6.8 was determined at room temperature with the help of magnetic stirrer.

Approximate solubility of drug was indicated from the following limits:

Very soluble: 1 part of the substance is soluble less than 1 part of the solvent.

Freely soluble: 1 part of the substance is soluble in 1 to 10 parts of the solvent.

Soluble: 1 part of the substance is soluble in 10 to 30 parts of the solvent.

Sparingly soluble: 1 part of the substance is soluble in 30 to 100 parts of the solvent.

Slightly soluble: 1 part of the substance is soluble in 100 to 1,000 parts of the solvent.

Very slightly soluble: 1 part of the substance is soluble in 1000 to 10,000 parts of the solvent.

Practically insoluble or insoluble: More than 10,000 parts of the solvent is required to dissolve 1 part of substance[74].

B) Melting Point

Melting point determination was done by using melting point apparatus. Small amount of pure drug of Metformin HCl was taken in a capillary tube and it was kept in the melting point apparatus and the melting point was noted[75].

Drug – excipients interaction studies:

FT-IR spectra were taken for the dried samples using FT-IR 8400S (Shimadzu, Japan) to determine the possible interactions between the drug and polymers. The plain drug, individual lecithin and cholesterol, combination of drug with cholesterol and lecithin in three different ratio (1:1, 1:2 and 1:3) were taken and mixed with KBr.

The samples were compressed to form a pellet using a hydraulic press. The prepared pellets were transformed into disk. The disk was applied to the centre of the sample holding device and scanned from 4,500 to 400 cm-1 using FT-IR spectrophotometer[76].

Formulation of liposomes loaded with Metformin hydrochloride:

The formulation of liposomes loaded with Metformin HCl was prepared by two different techniques namely, physical dispersion method and ether injection method. In both the techniques ratio of cholesterol was kept as same and the lecithin concentration was increased as 1:1, 1:2 and 1:3.

Physical dispersion method:

Liposomes were prepared by physical dispersion method using different ratio of soya lecithin and cholesterol was kept as constant. In this method the soya lecithin and cholesterol were dissolved in chloroform. Then it was spread over flat bottom conical flask and allowed to evaporate at room temperature for overnight without disturbing the solution for a formation of lipid film. The drug was dissolved in phosphate buffer pH 6.8. It act as an aqueous medium. Then the aqueous medium was added to the lipid film for hydration. For this the flask was inclined to one side and aqueous medium was introduced down the side of flask and flask was slowly returned to upright orientation. Then the conical flask was kept on water bath and the temperature was maintained at 37± 2ºC for 2 hours for the completion of hydration. The conical flask was gently shaken until the lipid layer was removed from wall of conical flask and formation a liposomes suspension. Then the formed liposomes suspension was stored at 4ºC for one day for the maturation of liposomes. The prepared liposome suspension was centrifuged at 15,000 rpm for 20 mins. Then the precipitate was collected and diluted with distilled water for further studies[35]. Different batches of liposomes were prepared as per the general method described above and composition for the preparation of liposomes is given in Table 5.

Ether injection method:

Liposomes were prepared by ether injection method using different ratio of soya lecithin and cholesterol was kept as constant. In this method the cholesterol and soya lecithin were dissolved in ether and methanol. The drug was dissolved in phosphate buffer pH 6.8. It act as an aqueous medium. The aqueous medium was heated to 60°C. The method involves injecting drop by drop of ether-lipid solutions into the above warmed aqueous medium. The ether vaporizes upon contacting the aqueous phase, and the dispersed lipid forms primarily unilamellar liposomes. Then the product was collected and it was stored at 4°C for maturation of liposome. Then prepared liposomal suspension was centrifuged at 15,000 rpm for 20 mins. The precipitate was diluted with distilled water for evaluation studies[77]. Different batches of liposomes were prepared as per the general method described above and composition for the preparation of liposomes is given in **Table No. 4**.

Table No. 4 Formulation of Metformin HCl liposomes

S. No.	Ingredients	Physical dispersion method			Ether injection method		
		F 1	F 2	F 3	F 4	F 5	F 6
1.	Cholesterol	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
2.	Lecithin	100 mg	200 mg	300 mg	100 mg	200 mg	300 mg
3.	Metformin HCl	10 gm	10 gm	10 gm	10 gm	10 gm	10 gm
4.	Ether	-	-	-	7 ml	7 ml	7 ml
5.	Methanol	-	-	-	3 ml	3 ml	3 ml
6	Chloroform	5 ml	5 ml	5 ml	-	-	-
7.	Phosphate buffer pH 6.8	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml

EVALUATION OF LIPOSOMES:

1. Determination of percentage drug entrapment efficiency:

Drug entrapment efficiency was calculated by using centrifugation method. 10 ml of liposome suspension was taken and centrifuged at 15,000 rpm for 20 mins. The supernatant liquid was collected and suitably diluted. Then the absorbance was taken at 233 nm with the help of UV double beam spectrophotometer using pH 6.8 as a blank. The drug entrapment efficiency was calculated from the following formula[78].

$$\text{Total entrapment efficiency} = \frac{\text{Amount of drug in supernatant liquid}}{\text{Amount of drug}} \times 100$$

2. Morphology analysis:

The prepared Metformin HCl liposomes for all the formulations were viewed under for observing the vesicle formation and discreteness of dispersed vesicles. A slide was prepared by placing a drop of liposome dispersion on a glass slide and cover slip was placed over it and this slide was viewed under optical microscope at 40X magnification. Photographs were taken to prepared slides using digital camera[33].

3. *In vitro* drug release study:

Apparatus : USP TYPE II (Paddle)

RPM : 50

Temperature : 37ºC ± 0.5ºC

Time : 30 min. interval Upto 8 hrs

The *in vitro* release for all the formulated Metformin HCl liposomes were carried out for 8 hours in phosphate buffer p H 6.8. The studies were carried in USP dissolution apparatus II (Paddle) at 37ºC ± 0.5ºC and 50 rpm speed. 900 ml of phosphate buffer p H 6.8 was used as a dissolution medium. Equivalent to 100 mg of Metformin HCl liposome was taken in a dissolution jar contains dissolution medium and the paddle was rotated at 50 rpm. 1 ml of samples were withdrawn at every 30 min. upto 480 mins and make upto 10 ml with pH 6.8 and analyzed for Metformin HCl content at 233 nm with pH 6.8 as blank using double beam UV double beam spectrophotometer[79].

4. Particle size determination:

The particle size determination is done by using Malven particle size analyzer. Groups of particles are dispersed in a liquid medium and measured as they are circulated between the flow cell, which is placed in the measurement unit, and a dispersion bath in the sampler. The dispersion bath incorporates a stirrer and an ultrasonic sonicator. A pump delivers the dispersed suspension to the flow cell. The pump is specially designed to ensure both liquid medium and the particles are circulated. It can be controlled from a PC. Organic solvents can be used as dispersion media[80].

5. Stability studies:

The behavior of the liposome to retain the drug was studied by storing the liposome at two different temperature conditions, i.e., 4ºC (refrigerator RF), 25°C±2ºC for a period of 1 month. The liposomal preparations were kept in sealed vials. At 30th day the samples were analyzed for the drug content following the same method described in % drug encapsulation efficiency and *in vitro* drug release. And also the liposomes were studied for their morphology[81].

RESULT AND DISSCUSSION

RESULTS AND DISCUSSION

The research study was aimed to formulate Metformin HCl liposomes to sustain the action of drug for over the period of 8 hours. The liposomes were prepared by physical dispersion method and ether injection method. Soya lecithin and cholesterol were used for encapsulating the drug and also to release the drug in sustained manner. Chloroform, ether and methanol were used as a solvent. Phosphate buffer pH 6.8 was used as a hydration medium for loading the drug.

Preformulation studies such like solubility analysis, melting point and FT – IR studies were carryout before the formulations. After formulation, the liposomes were evaluated for various parameters like percentage drug entrapment efficiency, microscopic analysis, particle size analysis, *in vitro* drug release studies and stability study.

PREFORMULATION STUDIES

A) Solubility

The drug should be dissolve in solvents and also dissolution medium so the solubility analysis for the drug was important. The solubility of raw drug was determined by dissolving in distilled water, methanol and phosphate buffer pH 6.8. The drug was found to be freely soluble in water, soluble in methanol and phosphate buffer p H 6.8.

B) Melting point

The melting point was confirmed the Metformin HCl present in raw material of drug. It was found to be 224ºC within the specification range. So it confirmed Metformin HCl present in raw material of drug.

C) DRUG – EXCIPIENTS INTERACTION STUDIES:

The FT – IR studies of pure Metformin HCl, cholesterol, soya lecithin and Metformin HCl+ cholesterol + soya lecithin were conduct to study the interaction between the drug and excipients.

IR spectral analysis showed that the fundamental peaks and patterns of the spectra were similar both in pure drug and combination containing drug and highest proportion of excipients. This indicated that there was no chemical interaction between Metformin HCl and the other excipients used in the formulations. The spectral datas are presented in **Table No. 5 - 8** and spectral peaks were presented graphically in **Figure No. 5 – 9.**

FT – IR SPECTRUM OF PURE METFORMIN HCL

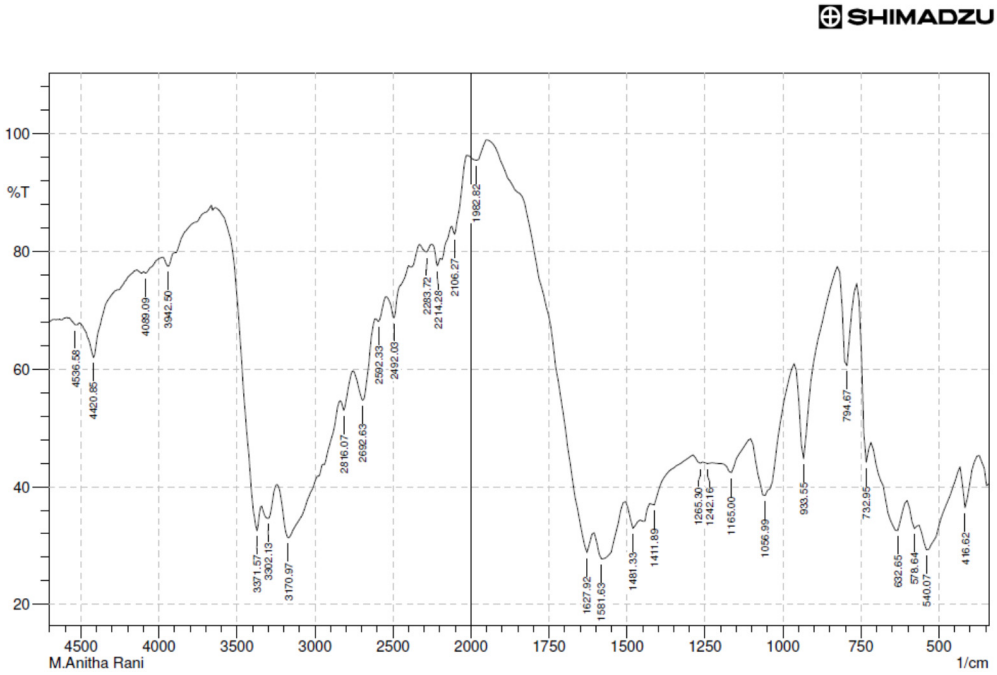


Figure No. 5 FT – IR Spectrum of pure Metformin HCl

Table No. 5 FT – IR Spectrum of pure Metformin HCl

Wave length (cm^{-1})	Functional group
3372	N-H stretching
1582	Amino N-H bending
1466	CH_3 bending alkanes
1057	C-N Stretching
957	Alkene C-H bending

FT – IR SPECTRUM OF CHOLESTEROL

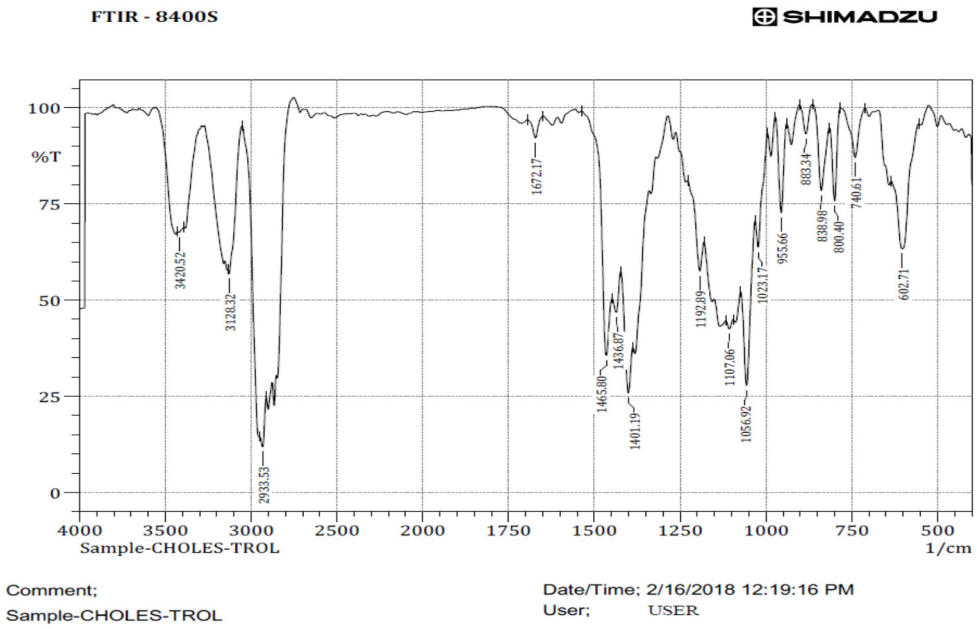


Figure No. 6

Table No. 6 FT – IR Spectrum of cholesterol

Wave length(cm^{-1})	Functional group
3421	N-H stretching
1466	CH_3 bending alkanes
1057	C-N Stretching
955	Alkene C-H bending

FT – IR SPECTRUM OF SOYA LECITHIN

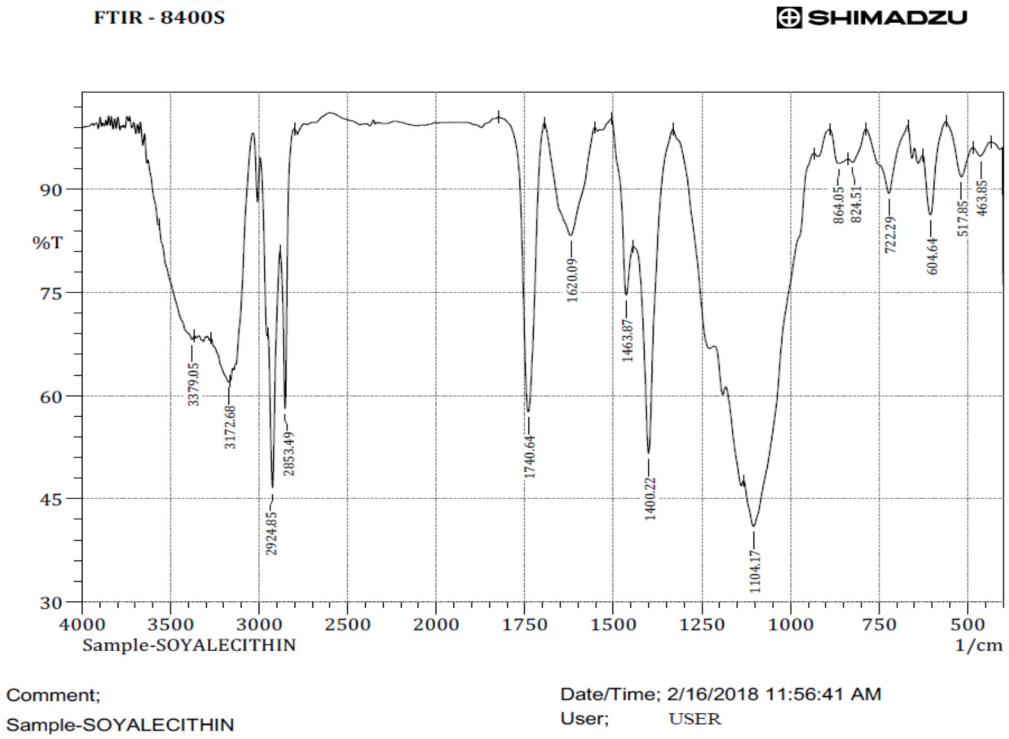


Figure No. 7

Table No. 7 FT – IR Spectrum of soya lecithin

Wave length (cm^{-1})	Functional group
3379	N-H stretching
1620	Amino N-H bending
1464	CH_3 bending alkanes
1104	C-N Stretching
864	Alkene C-H bending

FT – IR SPECTRUM OF COMBINATION OF METFORMIN HCl, CHOLESTEROL AND SOYA LECITHIN

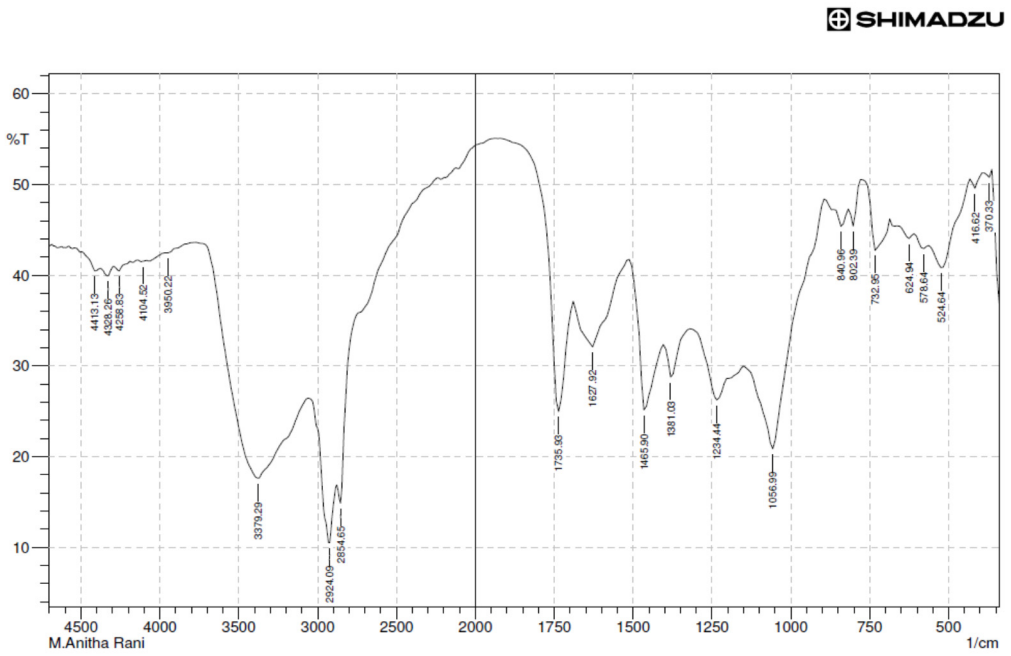


Figure No. 8

Table No. 7 FT – IR Spectrum of combination of Metformin HCl + cholesterol + soya lecithin

Wave length (cm^{-1})	Functional group
3372	N-H stretching
1582	Amino N-H bending
1466	CH_3 bending alkanes
1057	C-N Stretching
957	Alkene C-H bending

Table No. 8

FT – IR Spectrum of pure Metformin HCl, cholesterol, soya lecithin and combination of Metformin HCl + cholesterol + soya lecithin

Functional group	N-H stretching (cm^{-1})	Amino N-H bending (cm^{-1})	CH_3 bending alkanes (cm^{-1})	C-N Stretching (cm^{-1})	Alkene C-H bending (cm^{-1})
Drug	3372	1582	1466	1057	957
Cholesterol	3421	-	1466	1057	955
Soya lecithin	3379	1620	1464	1104	864
Combination of drug + cholesterol + soya lecithin	3372	1582	1466	1057	957

EVALUATON OF METORMIN HCL LIPOSOMES

Percentage drug entrapment efficiency

The percentage drug entrapment efficiency of liposomes were prepared by physical dispersion method and ether injection method. The formulations was formulated by varying the cholesterol – soya lecithin ratio. It was found to be that percentage drug entrapment efficiency of formulations F 1, F 2 and F 3 were 86.60 %, 79.90 % and 73.10 % respectively and formulations F 4, F 5 and F 6 were 30.47%, 39.58% and 39.69% respectively. The results may adjudge physical dispersion method have better drug entrapment efficiency than ether injection method.

Morphology analysis

The morphology characters of liposomes were analyzed by optical microscopy (Olympus Opto System, India) and the images were taken using digital camera. The formulation F 1, F 2, F 3, F4, F 5 and F 6 microscopic images were showed in **Figure No. 9 -14.**

Prepared liposomes F 1 to F 6 shows well identified morphology characters.

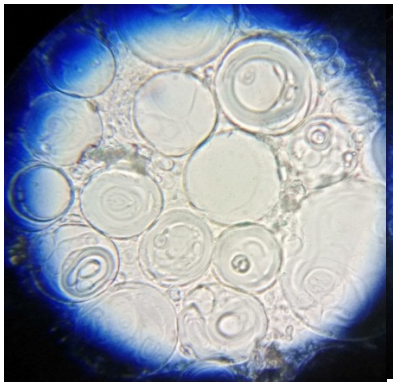


Figure No. 9 Microscopic image (45 X) of F 1 formulation

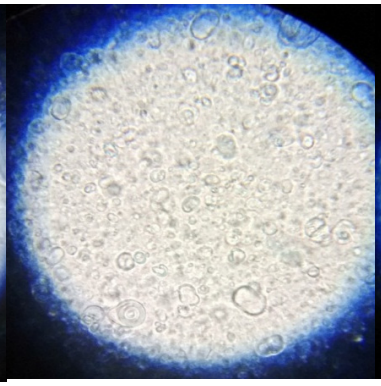


Figure No. 10 Microscopic image (45 X) of F 2 formulation

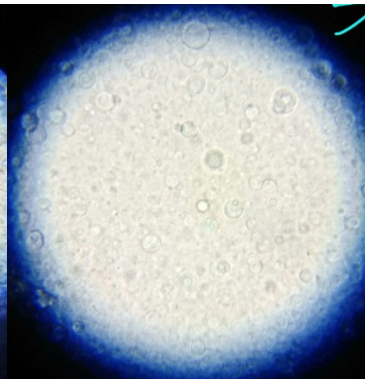


Figure No. 11 Microscopic image (45x) of F 3 formulation

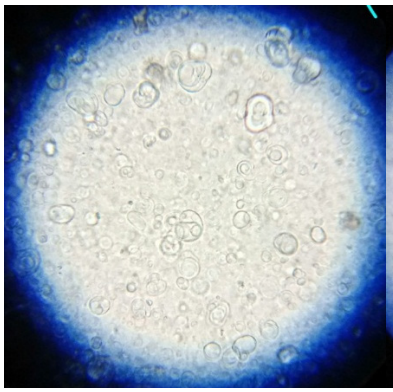


Figure No. 12 Microscopic image (45x) of F 4 formulation

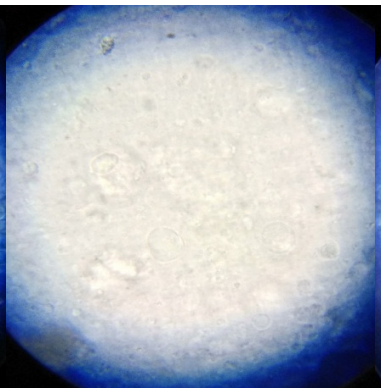


Figure No. 13 Microscopic image (45x) of F 5 formulation

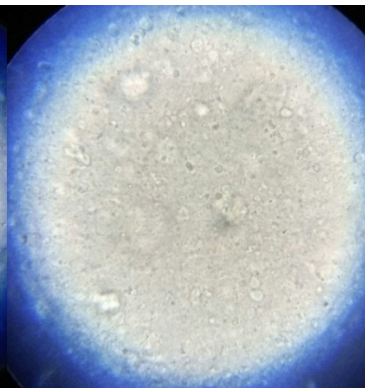


Figure No. 14 Microscopic image (45x) of F 6 formulation

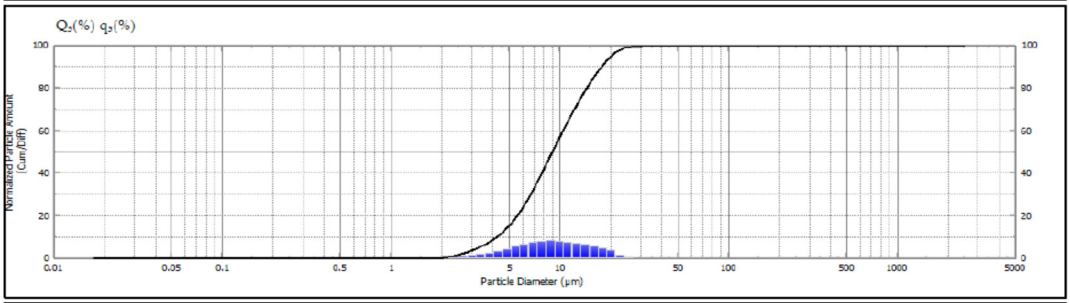
Particle size analysis

The particle size analysis was carried out by particle size analyzer for all the prepared liposome formulations. The particle size for all the formulated liposomes were found be in the range of 30.617 µm to 0.031µm as shown in **Table No. 7** and graphically showed in **Figure No. 15 to 20.** The particle size data showed that when the concentration of soya lecithin was increased the particle size was decreased for all the formulations of Metformin HCl liposomes in prepared by both methods. The particle size of Metformin HCl liposomes of F 3 and F 6 were found to be lower when compared with other formulations this may be due to higher concentration of soya lecithin.

Shimadzu SALD-2300(WingSALD II:Version 3.1.1)			Flow Cell with Sampler
File Name	Anitha 1 1 ppt	Meas Date	18/09/10 16:31:26
Sample ID	PPT (1:1)	Sample No.	4
Comment	Pharmacy		

Median D	8.917	Mean V	8.665
Modal D	9.316	Std Dev	0.239

25.000 %D(µm)	50.000 %D(µm)	75.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)
6.039	8.917	13.076	0.000	0.000	0.000	0.000	0.000	0.000



	Diam x(µm)	Cum% Q3(%)	Diff% q3(%)		Diam x(µm)	Cum% Q3(%)	Diff% q3(%)		Diam x(µm)	Cum% Q3(%)	Diff% q3(%)		Diam x(µm)	Cum% Q3(%)	Diff% q3(%)
1	2500.000	100.000	0.000	27	113.342	100.000	0.000	53	5.139	16.808	4.230	79	0.233	0.000	0.000
2	2219.551	100.000	0.000	28	100.628	100.000	0.000	54	4.562	12.578	3.273	80	0.207	0.000	0.000
3	1970.562	100.000	0.000	29	89.339	100.000	0.000	55	4.050	9.305	2.498	81	0.184	0.000	0.000
4	1749.505	100.000	0.000	30	79.317	100.000	0.000	56	3.596	6.807	1.916	82	0.163	0.000	0.000
5	1553.246	100.000	0.000	31	70.419	100.000	0.000	57	3.193	4.891	1.600	83	0.145	0.000	0.000
6	1379.004	100.000	0.000	32	62.520	100.000	0.000	58	2.834	3.291	1.251	84	0.129	0.000	0.000
7	1224.308	100.000	0.000	33	55.506	100.000	0.000	59	2.516	2.040	0.967	85	0.114	0.000	0.000
8	1086.965	100.000	0.000	34	49.280	100.000	0.000	60	2.234	1.074	0.655	86	0.101	0.000	0.000
9	965.030	100.000	0.000	35	43.752	100.000	0.000	61	1.984	0.419	0.279	87	0.090	0.000	0.000
10	856.773	100.000	0.000	36	38.843	100.000	0.000	62	1.761	0.139	0.079	88	0.080	0.000	0.000
11	760.661	100.000	0.000	37	34.486	100.000	0.000	63	1.563	0.060	0.033	89	0.071	0.000	0.000
12	675.330	100.000	0.000	38	30.617	100.000	0.168	64	1.388	0.027	0.000	90	0.063	0.000	0.000
13	599.572	100.000	0.000	39	27.183	99.832	0.725	65	1.232	0.027	0.000	91	0.056	0.000	0.000
14	532.312	100.000	0.000	40	24.133	99.107	1.782	66	1.094	0.027	0.000	92	0.050	0.000	0.000
15	472.597	100.000	0.000	41	21.426	97.325	3.683	67	0.971	0.027	0.000	93	0.044	0.000	0.000
16	419.582	100.000	0.000	42	19.023	93.642	4.942	68	0.862	0.027	0.000	94	0.039	0.000	0.000
17	372.513	100.000	0.000	43	16.889	88.700	5.901	69	0.766	0.027	0.007	95	0.035	0.000	0.000
18	330.725	100.000	0.000	44	14.994	82.799	6.681	70	0.680	0.019	0.016	96	0.031	0.000	0.000
19	293.624	100.000	0.000	45	13.312	76.118	7.192	71	0.604	0.003	0.003	97	0.027	0.000	0.000
20	260.685	100.000	0.000	46	11.819	68.926	7.676	72	0.536	0.000	0.000	98	0.024	0.000	0.000
21	231.442	100.000	0.000	47	10.493	61.249	8.161	73	0.476	0.000	0.000	99	0.022	0.000	0.000
22	205.479	100.000	0.000	48	9.316	53.088	8.292	74	0.422	0.000	0.000	100	0.019	0.000	0.000
23	182.428	100.000	0.000	49	8.271	44.796	8.233	75	0.375	0.000	0.000	101	0.017	0.000	0.000
24	161.963	100.000	0.000	50	7.343	36.563	7.685	76	0.333	0.000	0.000				
25	143.794	100.000	0.000	51	6.519	28.878	6.561	77	0.296	0.000	0.000				
26	127.664	100.000	0.000	52	5.788	22.317	5.509	78	0.262	0.000	0.000				

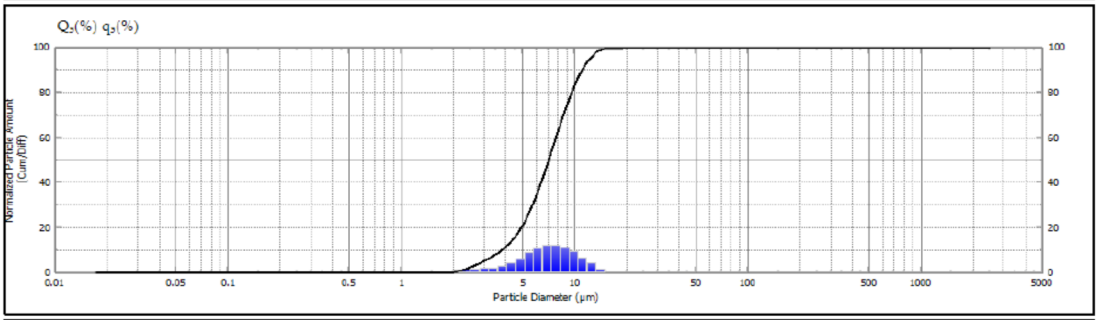
Condition File Name					
Ref Index	2.00-0.00i				
Decentralized Solvent			Dispersing Agent		
Denst of Disper Agent			Dispersion Method		
Pump Speed	5.0		Time for Sonicator	10	
Sample Preparation					
Distribution Function	None		Smoothing Level	0	
Select Emulation Table			Data Shift	0	
Sequence File Name					

Figure No. 15 Particle size range of F 1 formulation

Shimadzu SALD-2300(WingSALD II:Version 3.1.1)				Flow Cell with Sampler	
File Name	Anitha 1 2 pdm			Meas Date	18/09/10 16:25:48
Sample ID	PDM (1:2)		Sample No.	3	
Comment	Pharmacy				

Median D	7.005	Mean V	6.665
Modal D	7.343	Std Dev	0.182

25.000 %D(µm)	50.000 %D(µm)	75.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)
5.264	7.005	9.062	0.000	0.000	0.000	0.000	0.000	0.000



	Diam x(µm)	Cum% Q3(%)	Diff% q3(%)		Diam x(µm)	Cum% Q3(%)	Diff% q3(%)		Diam x(µm)	Cum% Q3(%)	Diff% q3(%)		Diam x(µm)	Cum% Q3(%)	Diff% q3(%)
1	2500.000	100.000	0.000	27	113.342	100.000	0.000	53	5.139	23.006	6.325	79	0.233	0.000	0.000
2	2219.551	100.000	0.000	28	100.628	100.000	0.000	54	4.562	16.681	4.555	80	0.207	0.000	0.000
3	1970.562	100.000	0.000	29	89.339	100.000	0.000	55	4.050	12.126	3.159	81	0.184	0.000	0.000
4	1749.505	100.000	0.000	30	79.317	100.000	0.000	56	3.596	8.968	2.281	82	0.163	0.000	0.000
5	1553.246	100.000	0.000	31	70.419	100.000	0.000	57	3.193	6.686	2.117	83	0.145	0.000	0.000
6	1379.004	100.000	0.000	32	62.520	100.000	0.000	58	2.834	4.569	1.741	84	0.129	0.000	0.000
7	1224.308	100.000	0.000	33	55.506	100.000	0.000	59	2.516	2.828	1.432	85	0.114	0.000	0.000
8	1086.965	100.000	0.000	34	49.280	100.000	0.000	60	2.234	1.396	0.998	86	0.101	0.000	0.000
9	965.030	100.000	0.000	35	43.752	100.000	0.000	61	1.984	0.398	0.345	87	0.090	0.000	0.000
10	856.773	100.000	0.000	36	38.843	100.000	0.000	62	1.761	0.053	0.042	88	0.080	0.000	0.000
11	760.661	100.000	0.000	37	34.486	100.000	0.000	63	1.563	0.011	0.011	89	0.071	0.000	0.000
12	675.330	100.000	0.000	38	30.617	100.000	0.000	64	1.388	0.000	0.000	90	0.063	0.000	0.000
13	599.572	100.000	0.000	39	27.183	100.000	0.000	65	1.232	0.000	0.000	91	0.056	0.000	0.000
14	532.312	100.000	0.000	40	24.133	100.000	0.000	66	1.094	0.000	0.000	92	0.050	0.000	0.000
15	472.597	100.000	0.000	41	21.426	100.000	0.000	67	0.971	0.000	0.000	93	0.044	0.000	0.000
16	419.582	100.000	0.000	42	19.023	100.000	0.043	68	0.862	0.000	0.000	94	0.039	0.000	0.000
17	372.513	100.000	0.000	43	16.889	99.957	0.134	69	0.766	0.000	0.000	95	0.035	0.000	0.000
18	330.725	100.000	0.000	44	14.994	99.823	1.463	70	0.680	0.000	0.000	96	0.031	0.000	0.000
19	293.624	100.000	0.000	45	13.312	98.360	4.150	71	0.604	0.000	0.000	97	0.027	0.000	0.000
20	260.685	100.000	0.000	46	11.819	94.210	6.524	72	0.536	0.000	0.000	98	0.024	0.000	0.000
21	231.442	100.000	0.000	47	10.493	87.686	9.431	73	0.476	0.000	0.000	99	0.022	0.000	0.000
22	205.479	100.000	0.000	48	9.316	78.254	11.158	74	0.422	0.000	0.000	100	0.019	0.000	0.000
23	182.428	100.000	0.000	49	8.271	67.096	12.307	75	0.375	0.000	0.000	101	0.017	0.000	0.000
24	161.963	100.000	0.000	50	7.343	54.790	12.183	76	0.333	0.000	0.000				
25	143.794	100.000	0.000	51	6.519	42.607	10.651	77	0.296	0.000	0.000				
26	127.664	100.000	0.000	52	5.788	31.956	8.950	78	0.262	0.000	0.000				

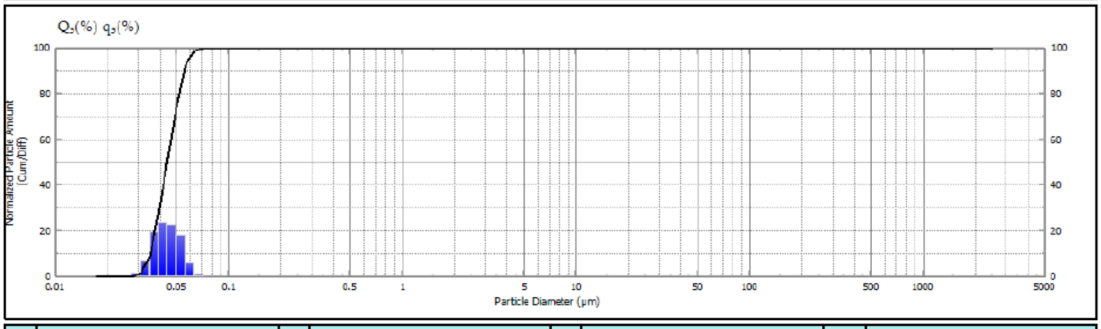
Condition File Name					
Ref Index	2.00-0.00i				
Decentralized Solvent			Dispersing Agent		
Denst of Disper Agent			Dispersion Method		
Pump Speed	5.0		Time for Sonicator	10	
Sample Preparation					
Distribution Function	None		Smoothing Level	0	
Select Emulation Table			Data Shift	0	
Sequence File Name					

Figure No. 16 Particle size range of F 2 formulation

Shimadzu SALD-2300(WingSALD II:Version 3.1.1)			Flow Cell with Sampler
File Name	M. Anitha Rani - 2	Meas Date	18/08/25 16:50:35
Sample ID	LiposomePDM	Sample No.	2
Comment	Metformin encapsulated by cholestrol		

Median D	0.044	Mean V	0.044
Modal D	0.039	Std Dev	0.081

25.000 %D(μm)	50.000 %D(μm)	75.000 %D(μm)	0.000 %D(μm)	0.000 %D(μm)	0.000 %D(μm)	0.000 %D(μm)	0.000 %D(μm)	0.000 %D(μm)
0.038	0.044	0.050	0.000	0.000	0.000	0.000	0.000	0.000



	Diam x(μm)	Cum% Q3(%)	Diff% q3(%)		Diam x(μm)	Cum% Q3(%)	Diff% q3(%)		Diam x(μm)	Cum% Q3(%)	Diff% q3(%)		Diam x(μm)	Cum% Q3(%)	Diff% q3(%)
1	2500.000	100.000	0.000	27	113.342	100.000	0.000	53	5.139	100.000	0.000	79	0.233	100.000	0.000
2	2219.551	100.000	0.000	28	100.628	100.000	0.000	54	4.562	100.000	0.000	80	0.207	100.000	0.000
3	1970.562	100.000	0.000	29	89.339	100.000	0.000	55	4.050	100.000	0.000	81	0.184	100.000	0.000
4	1749.505	100.000	0.000	30	79.317	100.000	0.000	56	3.596	100.000	0.000	82	0.163	100.000	0.000
5	1553.246	100.000	0.000	31	70.419	100.000	0.000	57	3.193	100.000	0.000	83	0.145	100.000	0.000
6	1379.004	100.000	0.000	32	62.520	100.000	0.000	58	2.834	100.000	0.000	84	0.129	100.000	0.000
7	1224.308	100.000	0.000	33	55.506	100.000	0.000	59	2.516	100.000	0.000	85	0.114	100.000	0.000
8	1086.965	100.000	0.000	34	49.280	100.000	0.000	60	2.234	100.000	0.000	86	0.101	100.000	0.000
9	965.030	100.000	0.000	35	43.752	100.000	0.000	61	1.984	100.000	0.000	87	0.090	100.000	0.000
10	856.773	100.000	0.000	36	38.843	100.000	0.000	62	1.761	100.000	0.000	88	0.080	100.000	0.000
11	760.661	100.000	0.000	37	34.486	100.000	0.000	63	1.563	100.000	0.000	89	0.071	100.000	0.936
12	675.330	100.000	0.000	38	30.617	100.000	0.000	64	1.388	100.000	0.000	90	0.063	99.064	6.068
13	599.572	100.000	0.000	39	27.183	100.000	0.000	65	1.232	100.000	0.000	91	0.056	92.996	18.281
14	532.312	100.000	0.000	40	24.133	100.000	0.000	66	1.094	100.000	0.000	92	0.050	74.716	22.780
15	472.597	100.000	0.000	41	21.426	100.000	0.000	67	0.971	100.000	0.000	93	0.044	51.936	23.506
16	419.582	100.000	0.000	42	19.023	100.000	0.000	68	0.862	100.000	0.000	94	0.039	28.430	19.491
17	372.513	100.000	0.000	43	16.889	100.000	0.000	69	0.766	100.000	0.000	95	0.035	8.940	7.157
18	330.725	100.000	0.000	44	14.994	100.000	0.000	70	0.680	100.000	0.000	96	0.031	1.783	1.783
19	293.624	100.000	0.000	45	13.312	100.000	0.000	71	0.604	100.000	0.000	97	0.027	0.000	0.000
20	260.685	100.000	0.000	46	11.819	100.000	0.000	72	0.536	100.000	0.000	98	0.024	0.000	0.000
21	231.442	100.000	0.000	47	10.493	100.000	0.000	73	0.476	100.000	0.000	99	0.022	0.000	0.000
22	205.479	100.000	0.000	48	9.316	100.000	0.000	74	0.422	100.000	0.000	100	0.019	0.000	0.000
23	182.428	100.000	0.000	49	8.271	100.000	0.000	75	0.375	100.000	0.000	101	0.017	0.000	0.000
24	161.963	100.000	0.000	50	7.343	100.000	0.000	76	0.333	100.000	0.000				
25	143.794	100.000	0.000	51	6.519	100.000	0.000	77	0.296	100.000	0.000				
26	127.664	100.000	0.000	52	5.788	100.000	0.000	78	0.262	100.000	0.000				

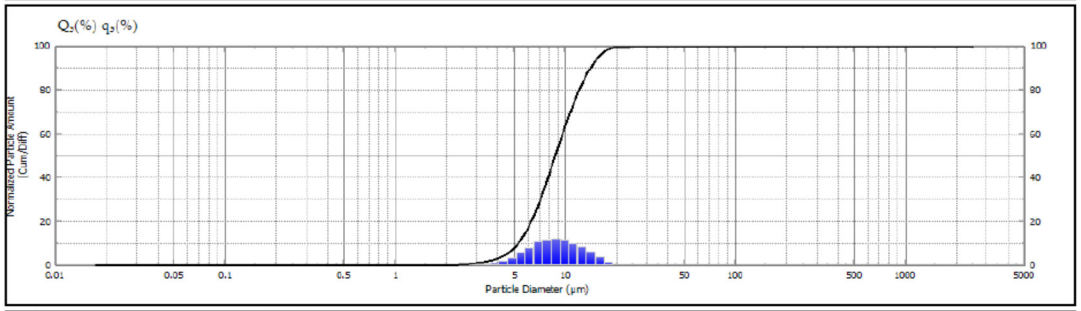
Condition File Name					
Ref Index	2.00-0.00i				
Decentralized Solvent			Dispersing Agent		
Denst of Disper Agent			Dispersion Method		
Pump Speed	5.0		Time for Sonicator	10	
Sample Preparation					
Distribution Function	None		Smoothing Level	0	
Select Emulation Table			Data Shift	0	
Sequence File Name					

Figure No. 17 Particle size range of F 3 formulation

Shimadzu SALD-2300(WingSALD II:Version 3.1.1)			Flow Cell with Sampler
File Name	Anitha	Meas Date	18/09/10 16:15:08
Sample ID	EIM (1:1)	Sample No.	1
Comment	Pharmacy		

Median D	8.633	Mean V	8.477
Modal D	9.316	Std Dev	0.171

25.000 %D(µm)	50.000 %D(µm)	75.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)
6.645	8.633	11.271	0.000	0.000	0.000	0.000	0.000	0.000



	Diam x(µm)	Cum% Q3(%)	Diff% q3(%)		Diam x(µm)	Cum% Q3(%)	Diff% q3(%)		Diam x(µm)	Cum% Q3(%)	Diff% q3(%)		Diam x(µm)	Cum% Q3(%)	Diff% q3(%)
1	2500.000	100.000	0.000	27	113.342	100.000	0.000	53	5.139	9.276	3.564	79	0.233	0.000	0.000
2	2219.551	100.000	0.000	28	100.628	100.000	0.000	54	4.562	5.712	2.062	80	0.207	0.000	0.000
3	1970.562	100.000	0.000	29	89.339	100.000	0.000	55	4.050	3.650	1.238	81	0.184	0.000	0.000
4	1749.505	100.000	0.000	30	79.317	100.000	0.000	56	3.596	2.412	0.665	82	0.163	0.000	0.000
5	1553.246	100.000	0.000	31	70.419	100.000	0.000	57	3.193	1.747	0.603	83	0.145	0.000	0.000
6	1379.004	100.000	0.000	32	62.520	100.000	0.000	58	2.834	1.144	0.442	84	0.129	0.000	0.000
7	1224.308	100.000	0.000	33	55.506	100.000	0.000	59	2.516	0.702	0.365	85	0.114	0.000	0.000
8	1086.965	100.000	0.000	34	49.280	100.000	0.000	60	2.234	0.338	0.246	86	0.101	0.000	0.000
9	965.030	100.000	0.000	35	43.752	100.000	0.000	61	1.984	0.092	0.083	87	0.090	0.000	0.000
10	856.773	100.000	0.000	36	38.843	100.000	0.000	62	1.761	0.009	0.007	88	0.080	0.000	0.000
11	760.661	100.000	0.000	37	34.486	100.000	0.000	63	1.563	0.002	0.002	89	0.071	0.000	0.000
12	675.330	100.000	0.000	38	30.617	100.000	0.000	64	1.388	0.000	0.000	90	0.063	0.000	0.000
13	599.572	100.000	0.000	39	27.183	100.000	0.000	65	1.232	0.000	0.000	91	0.056	0.000	0.000
14	532.312	100.000	0.000	40	24.133	100.000	0.043	66	1.094	0.000	0.000	92	0.050	0.000	0.000
15	472.597	100.000	0.000	41	21.426	99.957	0.323	67	0.971	0.000	0.000	93	0.044	0.000	0.000
16	419.582	100.000	0.000	42	19.023	99.634	1.592	68	0.862	0.000	0.000	94	0.039	0.000	0.000
17	372.513	100.000	0.000	43	16.889	98.042	4.071	69	0.766	0.000	0.000	95	0.035	0.000	0.000
18	330.725	100.000	0.000	44	14.994	93.971	6.127	70	0.680	0.000	0.000	96	0.031	0.000	0.000
19	293.624	100.000	0.000	45	13.312	87.844	8.333	71	0.604	0.000	0.000	97	0.027	0.000	0.000
20	260.685	100.000	0.000	46	11.819	79.511	10.029	72	0.536	0.000	0.000	98	0.024	0.000	0.000
21	231.442	100.000	0.000	47	10.493	69.482	11.621	73	0.476	0.000	0.000	99	0.022	0.000	0.000
22	205.479	100.000	0.000	48	9.316	57.862	12.141	74	0.422	0.000	0.000	100	0.019	0.000	0.000
23	182.428	100.000	0.000	49	8.271	45.720	11.878	75	0.375	0.000	0.000	101	0.017	0.000	0.000
24	161.963	100.000	0.000	50	7.343	33.843	10.647	76	0.333	0.000	0.000				
25	143.794	100.000	0.000	51	6.519	23.195	8.016	77	0.296	0.000	0.000				
26	127.664	100.000	0.000	52	5.788	15.179	5.904	78	0.262	0.000	0.000				

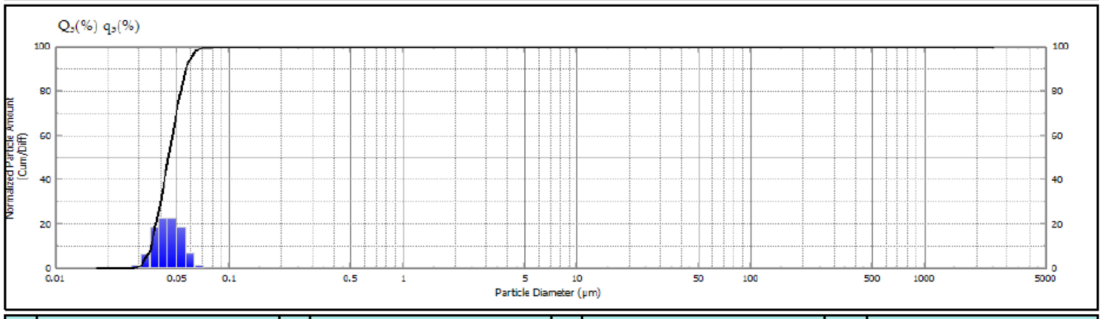
Condition File Name					
Ref Index	2.00-0.00i				
Decentralized Solvent			Dispersing Agent		
Denst of Disper Agent			Dispersion Method		
Pump Speed	5.0		Time for Sonicator	10	
Sample Preparation					
Distribution Function	None		Smoothing Level	0	
Select Emulation Table			Data Shift	0	
Sequence File Name					

Figure No. 18 Particle size range of F 4 formulation

Shimadzu SALD-2300(WingSALD II:Version 3.1.1)			Flow Cell with Sampler
File Name	Anitha 1 2	Meas Date	18/09/10 16:19:36
Sample ID	EIM (1:2)	Sample No.	2
Comment	Pharmacy		

Median D	0.044	Mean V	0.044
Modal D	0.050	Std Dev	0.083

25.000 %D(µm)	50.000 %D(µm)	75.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)	0.000 %D(µm)
0.038	0.044	0.051	0.000	0.000	0.000	0.000	0.000	0.000



	Diam x(µm)	Cum% Q3(%)	Diff% q3(%)		Diam x(µm)	Cum% Q3(%)	Diff% q3(%)		Diam x(µm)	Cum% Q3(%)	Diff% q3(%)		Diam x(µm)	Cum% Q3(%)	Diff% q3(%)
1	2500.000	100.000	0.000	27	113.342	100.000	0.000	53	5.139	100.000	0.000	79	0.233	100.000	0.000
2	2219.551	100.000	0.000	28	100.628	100.000	0.000	54	4.562	100.000	0.000	80	0.207	100.000	0.000
3	1970.562	100.000	0.000	29	89.339	100.000	0.000	55	4.050	100.000	0.000	81	0.184	100.000	0.000
4	1749.505	100.000	0.000	30	79.317	100.000	0.000	56	3.596	100.000	0.000	82	0.163	100.000	0.000
5	1553.246	100.000	0.000	31	70.419	100.000	0.000	57	3.193	100.000	0.000	83	0.145	100.000	0.000
6	1379.004	100.000	0.000	32	62.520	100.000	0.000	58	2.834	100.000	0.000	84	0.129	100.000	0.000
7	1224.308	100.000	0.000	33	55.506	100.000	0.000	59	2.516	100.000	0.000	85	0.114	100.000	0.000
8	1086.965	100.000	0.000	34	49.280	100.000	0.000	60	2.234	100.000	0.000	86	0.101	100.000	0.000
9	965.030	100.000	0.000	35	43.752	100.000	0.000	61	1.984	100.000	0.000	87	0.090	100.000	0.000
10	856.773	100.000	0.000	36	38.843	100.000	0.000	62	1.761	100.000	0.000	88	0.080	100.000	0.023
11	760.661	100.000	0.000	37	34.486	100.000	0.000	63	1.563	100.000	0.000	89	0.071	99.977	1.816
12	675.330	100.000	0.000	38	30.617	100.000	0.000	64	1.388	100.000	0.000	90	0.063	98.160	7.006
13	599.572	100.000	0.000	39	27.183	100.000	0.000	65	1.232	100.000	0.000	91	0.056	91.154	18.747
14	532.312	100.000	0.000	40	24.133	100.000	0.000	66	1.094	100.000	0.000	92	0.050	72.407	22.792
15	472.597	100.000	0.000	41	21.426	100.000	0.000	67	0.971	100.000	0.000	93	0.044	49.615	22.658
16	419.582	100.000	0.000	42	19.023	100.000	0.000	68	0.862	100.000	0.000	94	0.039	26.958	18.520
17	372.513	100.000	0.000	43	16.889	100.000	0.000	69	0.766	100.000	0.000	95	0.035	8.438	6.792
18	330.725	100.000	0.000	44	14.994	100.000	0.000	70	0.680	100.000	0.000	96	0.031	1.645	1.645
19	293.624	100.000	0.000	45	13.312	100.000	0.000	71	0.604	100.000	0.000	97	0.027	0.000	0.000
20	260.685	100.000	0.000	46	11.819	100.000	0.000	72	0.536	100.000	0.000	98	0.024	0.000	0.000
21	231.442	100.000	0.000	47	10.493	100.000	0.000	73	0.476	100.000	0.000	99	0.022	0.000	0.000
22	205.479	100.000	0.000	48	9.316	100.000	0.000	74	0.422	100.000	0.000	100	0.019	0.000	0.000
23	182.428	100.000	0.000	49	8.271	100.000	0.000	75	0.375	100.000	0.000	101	0.017	0.000	0.000
24	161.963	100.000	0.000	50	7.343	100.000	0.000	76	0.333	100.000	0.000				
25	143.794	100.000	0.000	51	6.519	100.000	0.000	77	0.296	100.000	0.000				
26	127.664	100.000	0.000	52	5.788	100.000	0.000	78	0.262	100.000	0.000				

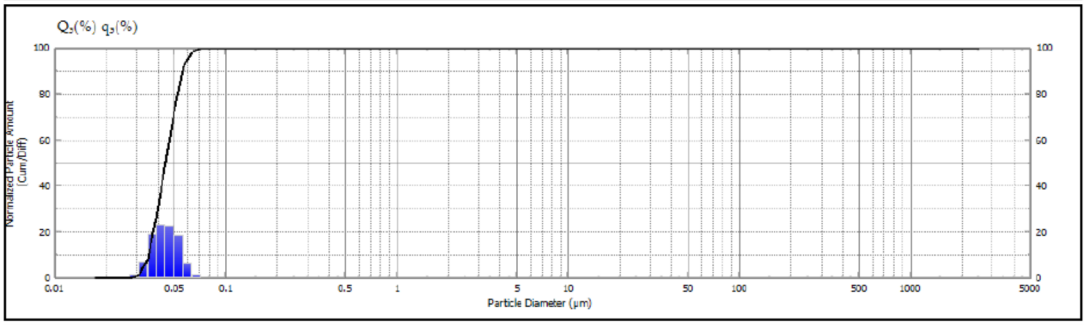
Condition File Name					
Ref Index	2.00-0.00i				
Decentralized Solvent			Dispersing Agent		
Denst of Disper Agent			Dispersion Method		
Pump Speed	5.0		Time for Sonicator	10	
Sample Preparation					
Distribution Function	None		Smoothing Level	0	
Select Emulation Table			Data Shift	0	
Sequence File Name					

Figure No. 19 Particle size range of F 5 formulation

Shimadzu SALD-2300(WingSALD II:Version 3.1.1)			Flow Cell with Sampler
File Name	M. Anitha Rani	Meas Date	18/08/25 16:30:49
Sample ID	Liposome EIM	Sample No.	1
Comment	Metformin encapsulated by cholestrol		

Median D	0.044	Mean V	0.044
Modal D	0.039	Std Dev	0.082

25.000 %D(μm)	50.000 %D(μm)	75.000 %D(μm)	0.000 %D(μm)	0.000 %D(μm)	0.000 %D(μm)	0.000 %D(μm)	0.000 %D(μm)	0.000 %D(μm)
0.038	0.044	0.051	0.000	0.000	0.000	0.000	0.000	0.000



	Diam x(μm)	Cum% Q3(%)	Diff% q3(%)		Diam x(μm)	Cum% Q3(%)	Diff% q3(%)		Diam x(μm)	Cum% Q3(%)	Diff% q3(%)		Diam x(μm)	Cum% Q3(%)	Diff% q3(%)
1	2500.000	100.000	0.000	27	113.342	100.000	0.000	53	5.139	100.000	0.000	79	0.233	100.000	0.000
2	2219.551	100.000	0.000	28	100.628	100.000	0.000	54	4.562	100.000	0.000	80	0.207	100.000	0.000
3	1970.562	100.000	0.000	29	89.339	100.000	0.000	55	4.050	100.000	0.000	81	0.184	100.000	0.000
4	1749.505	100.000	0.000	30	79.317	100.000	0.000	56	3.596	100.000	0.000	82	0.163	100.000	0.000
5	1553.246	100.000	0.000	31	70.419	100.000	0.000	57	3.193	100.000	0.000	83	0.145	100.000	0.000
6	1379.004	100.000	0.000	32	62.520	100.000	0.000	58	2.834	100.000	0.000	84	0.129	100.000	0.000
7	1224.308	100.000	0.000	33	55.506	100.000	0.000	59	2.516	100.000	0.000	85	0.114	100.000	0.000
8	1086.965	100.000	0.000	34	49.280	100.000	0.000	60	2.234	100.000	0.000	86	0.101	100.000	0.000
9	965.030	100.000	0.000	35	43.752	100.000	0.000	61	1.984	100.000	0.000	87	0.090	100.000	0.000
10	856.773	100.000	0.000	36	38.843	100.000	0.000	62	1.761	100.000	0.000	88	0.080	100.000	0.000
11	760.661	100.000	0.000	37	34.486	100.000	0.000	63	1.563	100.000	0.000	89	0.071	100.000	1.418
12	675.330	100.000	0.000	38	30.617	100.000	0.000	64	1.388	100.000	0.000	90	0.063	98.582	6.585
13	599.572	100.000	0.000	39	27.183	100.000	0.000	65	1.232	100.000	0.000	91	0.056	91.996	18.544
14	532.312	100.000	0.000	40	24.133	100.000	0.000	66	1.094	100.000	0.000	92	0.050	73.452	22.793
15	472.597	100.000	0.000	41	21.426	100.000	0.000	67	0.971	100.000	0.000	93	0.044	50.659	23.040
16	419.582	100.000	0.000	42	19.023	100.000	0.000	68	0.862	100.000	0.000	94	0.039	27.619	18.956
17	372.513	100.000	0.000	43	16.889	100.000	0.000	69	0.766	100.000	0.000	95	0.035	8.663	6.956
18	330.725	100.000	0.000	44	14.994	100.000	0.000	70	0.680	100.000	0.000	96	0.031	1.707	1.707
19	293.624	100.000	0.000	45	13.312	100.000	0.000	71	0.604	100.000	0.000	97	0.027	0.000	0.000
20	260.685	100.000	0.000	46	11.819	100.000	0.000	72	0.536	100.000	0.000	98	0.024	0.000	0.000
21	231.442	100.000	0.000	47	10.493	100.000	0.000	73	0.476	100.000	0.000	99	0.022	0.000	0.000
22	205.479	100.000	0.000	48	9.316	100.000	0.000	74	0.422	100.000	0.000	100	0.019	0.000	0.000
23	182.428	100.000	0.000	49	8.271	100.000	0.000	75	0.375	100.000	0.000	101	0.017	0.000	0.000
24	161.963	100.000	0.000	50	7.343	100.000	0.000	76	0.333	100.000	0.000				
25	143.794	100.000	0.000	51	6.519	100.000	0.000	77	0.296	100.000	0.000				
26	127.664	100.000	0.000	52	5.788	100.000	0.000	78	0.262	100.000	0.000				

Condition File Name					
Ref Index	2.00-0.00i				
Decentralized Solvent			Dispersing Agent		
Denst of Disper Agent			Dispersion Method		
Pump Speed	5.0		Time for Sonicator	10	
Sample Preparation					
Distribution Function	None			Smoothing Level	0
Select Emulation Table				Data Shift	0
Sequence File Name					

Figure No. 20 Particle size range of F 6 formulation

Table No. 9 Particle size of all the formulations of Metformin HCl liposomes

S. No.	Formulations	Particle size range
1.	F 1	30.617 – 1.563 µm
2.	F 2	19.023 – 1.563 µm
3.	F 3	0.071 -0.031µm
4.	F 4	24.133 – 1.563 µm
5.	F 5	0.081 – 0.031 µm
6.	F 6	0.071 -0.031µm

In vitro drug release studies

In vitro release studies were performed to evaluate the release of drug from the prepared Metformin HCl liposomes. The result of the *in vitro* release studies of all formulation were presented in **Table No. 10.**

Table No. 10 Cumulative percentage drug released of Metformin HCl from liposomes

S. No	Time (Mins)	F – 1	F - 2	F - 3	F – 4	F - 5	F – 6
1.	30	9.31±0.94	8.92±0.52	8.16±0.63	8.64±0.48	7.53±0.58	2.74±0.33
2.	60	15.76±0.59	12.19±0.61	11.24±0.80	16.63±0.67	13.73±0.37	5.34±0.94
3.	90	24.47±1.13	18.60±1.72	15.84±1.26	24.16±1.28	19.19±0.94	9.79±1.27
4.	120	32.70±2.54	25.22±1.47	20.78±2.42	31.48±1.88	26.48±0.71	15.75±0.57
5.	150	40.48±2.20	30.44±3.18	26.11±2.36	39.54±2.12	33.31±0.48	21.56±0.95
6.	180	45.49±1.85	36.58±3.54	30.60±2.44	47.31±2.30	38.18±0.43	26.58±0.42
7.	210	50.25±1.90	41.20±3.80	35.19±2.47	55.12±2.44	42.81±1.27	30.09±0.97
8.	240	57.43±1.72	47.65±3.87	38.49±2.61	62.44±2.32	47.44±2.58	33.31±1.51
9.	270	65.91±1.45	53.68±3.55	43.26±2.61	69.45±2.12	52.66±1.57	37.28±1.57
10.	300	74.00±3.11	58.45±3.00	46.98±2.38	76.56±1.47	57.29±1.36	42.40±2.03
11.	330	81.77±2.78	63.71±3.21	50.45±2.37	83.81±1.64	61.95±1.91	46.19±2.00
12.	360	88.04±2.81	69.84±3.56	55.85±2.37	91.06±1.57	66.51±1.36	51.41±1.88
13.	390	92.43±2.07	75.66±3.03	62.30±2.37	97.61±1.86	73.10±0.10	57.40±1.75
14.	420	95.83±2.11	80.70±2.63	69.23±2.51	100.58±1.58	79.13±1.61	64.06±1.55
15.	450	99.76±2.02	86.52±3.09	75.36±2.51	-	82.08±1.66	71.34±1.40
16.	480	103.03±2.47	91.92±2.72	82.12±2.51	-	85.06±1.73	79.05±1.03

All the values expressed as mean± standard deviation, n = 3

The Metformin HCl liposomes were prepared by physical dispersion method and ether injection method using different ratio of cholesterol and soya lecithin. The cumulative percentage drug release was compared with different formulations.

The cumulative percentage drug release of formulations F 1, F 2 and F 3 were found to be 103.03±2.47, 91.92±2.72 and 82.12±2.51 respectively in 8 hours. The

formulation F 1 show faster release than formulations F 2 and F 3 due to the lower concentration of soya lecithin.

The cumulative percentage drug release of formulations F 4 was found to be 100.58 ± 1.58 at the end of 7 hours. And the cumulative percentage drug release of formulations F 5 and F 6 were found to 85.06±1.73 and 81.39±1.12 respectively in 8 hours. The formulation F 4 show faster release than formulations F 5 and F 6. While the concentration of soya lecithin was increase it decrease the release of drug.

The prepared liposomes F 1 to F 6 showed sustained release of drug. When increased ratio of soya lecithin also sustain the release of drug was increased in both method of preparations.

The **Figure No. 21 and 22** shows the formulation F 1, F 2 and F 3 and F 4, F 5 and F 6 respectively in 8 hours.

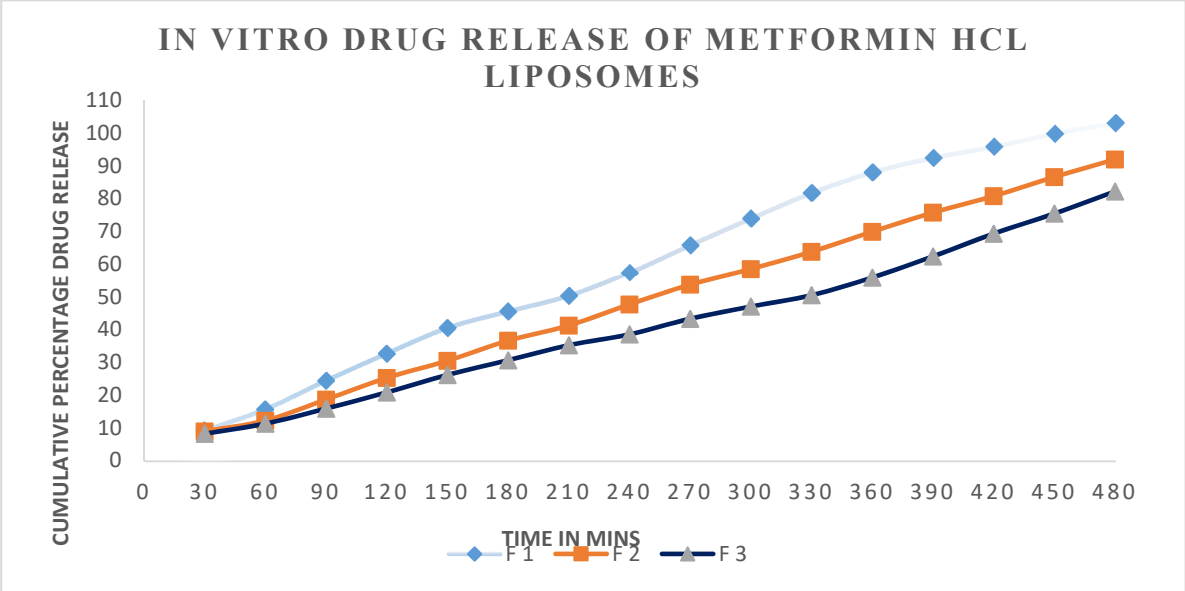


Figure No. 21 Comparative cumulative percentage drug release of Metformin HCl liposome formulations of F 1, F 2 and F 3

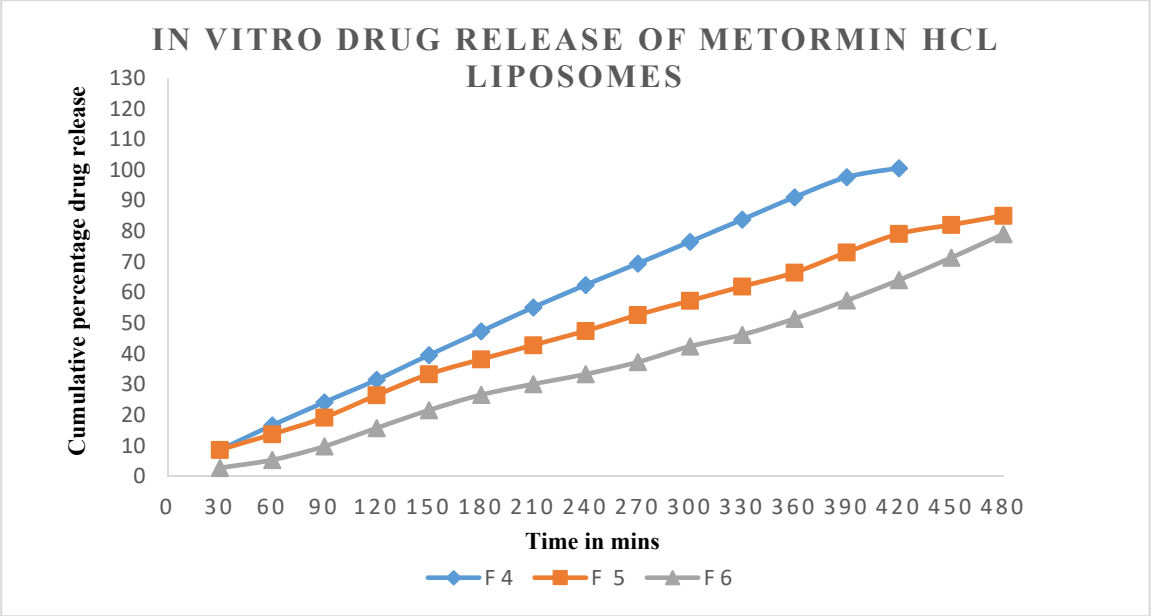


Figure No. 22 Comparative cumulative percentage drug release of Metformin HCl liposome formulations of F 4, F 5 and F 6

STABILITY STUDIES

All the formulations of Metformin HCl liposomes were relatively stable at 4ºC storage condition. The drug leakage percent amounts of original entrapped in liposomes were very small and the amount retained in vesicle had no significant difference after one month as compared to the amount immediately after preparation. But at the storage condition of 25°C±2°C, all the formulations of Metformin HCl liposomes were unstable. In addition, the result of drug entrapment studies showed higher leakage at higher temperature. This may be due the higher fluidity of lipid bilayer at higher temperature, resulting into higher drug leakage.

Table No. 11 Stability study of percentage drug entrapment of liposomes Metformin HCl liposomes compared with percentage drug entrapment of immediately after preparation.

S. No.	Formulations code	Immediately after preparation (%)	After one month	
			At 4°C	At 25°C±2°C
1.	**F 1**	86.60	85.92%	76.87%
2.	**F 2**	79.90 %	77.99%	70.98%
3.	**F 3**	73.10 %	72.08%	66.89%
4.	**F 4**	30.47%	29.35%	24.89%
5.	**F 5**	39.58%	38.44%	35.39%
6.	**F 6**	39.69%	38.36%	36.69%

The morphological characters of Metformin HCl liposomes for **F 1 – F 4** didn't show any characteristic changes after it was stored at 4ºC and 25ºC±2ºC for a period of one month. F 5 and F 6 formulations were showed slightly reduced in the size after it was stored at 25ºC±2ºC for a period of one month but there was no changes for the same formulation when it was stored at 4ºC. Microscopic images of all the formulations (F 1 – F 6) of Metformin HCl liposomes were compared with before and after stability stability studies were shown in **Figure N**o. **23 – 40**.

Morphology characters of liposomes – Before and after stability study

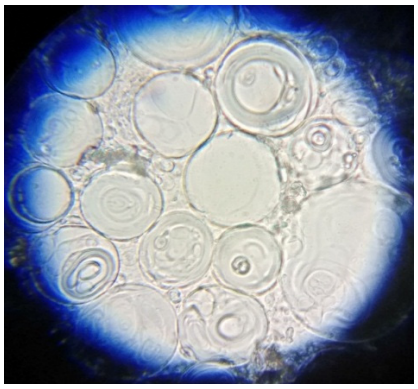


Figure No. 23 F 1, Immediately after preparation (45x)

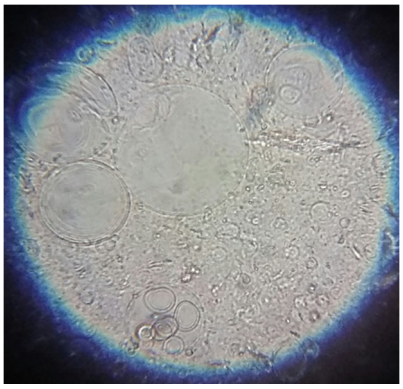


Figure No. 24 F 1, After stability study at 4ºC (45x)

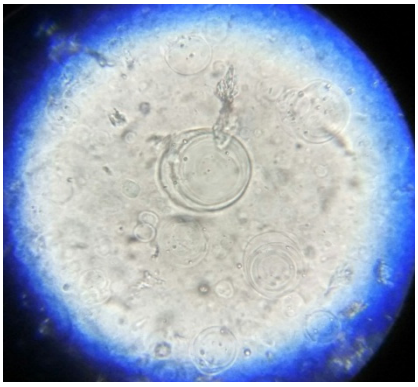


Figure No. 25 F 1, After stability study at 25ºC±2ºC (45x)

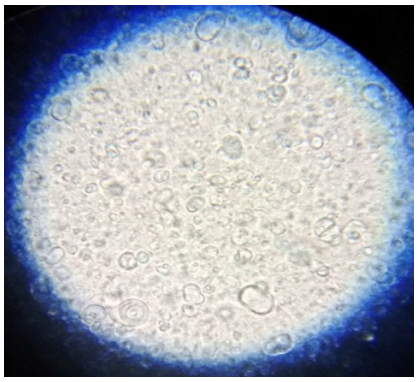


Figure No. 26 F 2 Immediately after preparation (45x)

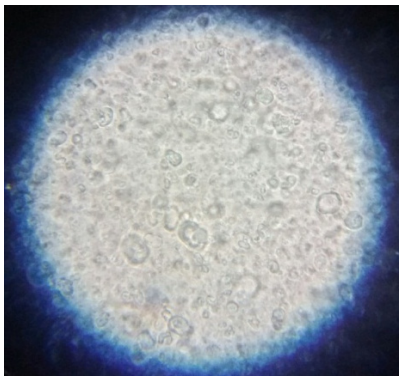


Figure No. 27 F 2, After stability study at 4ºC (45x)

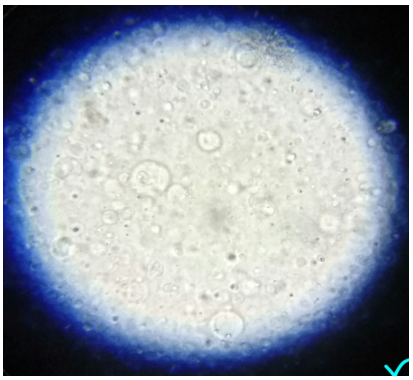


Figure No.28 F 2, After stability study at 25ºC±2ºC (45x)

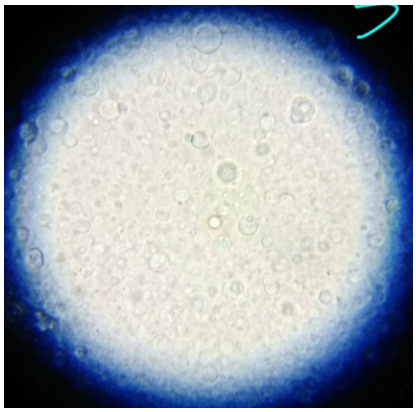


Figure No. 29 F 3, Immediately after preparation (45x)

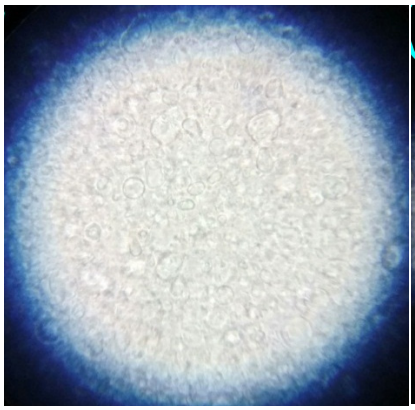


Figure No. 30 F 3, After stability study at 4ºC (45x)

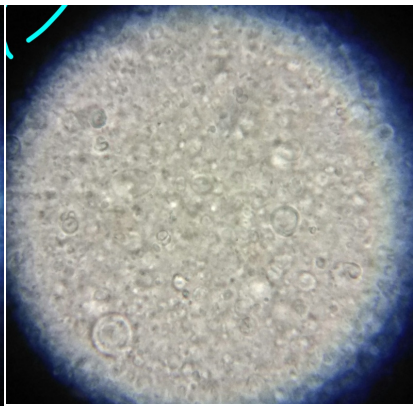


Figure No. 31 F 3, After stability study at 25ºC±2ºC (45x)

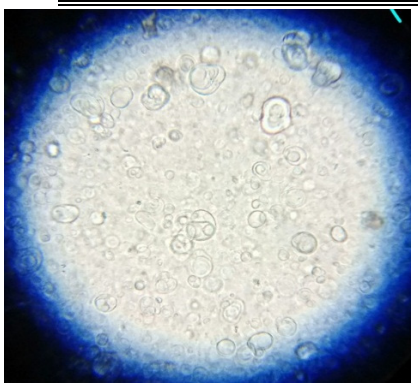


Figure No. 32 F 4, Immediately after preparation (45x)

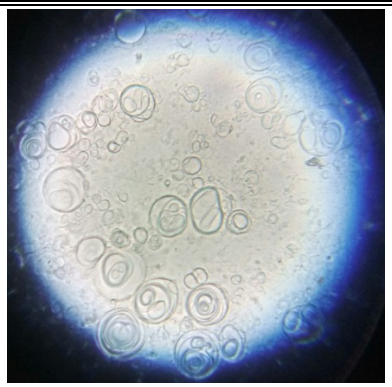


Figure No. 33 F 4, After stability study at 4ºC (45x)

Figure No. 34 F 4, After stability study at 25ºC±2ºC (45x)

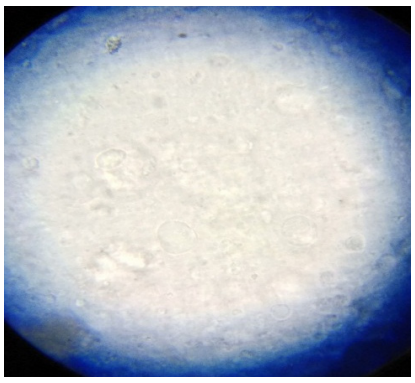


Figure No. 35 F 5, Immediately after preparation (45x)

Figure No. 36 F 5, After stability study at 4ºC (45x)

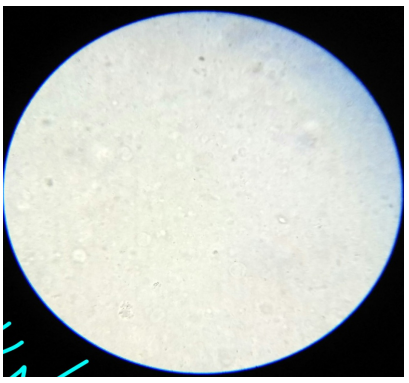


Figure No. 37 F 5, After stability study at 25ºC±2ºC (45x)

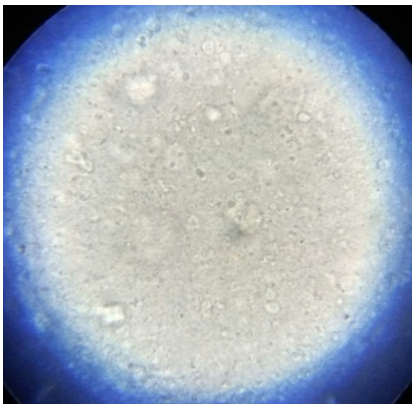


Figure No. 38 F 6, Immediately after preparation (45x)

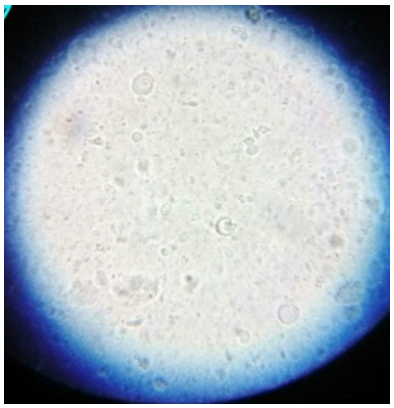


Figure No. 39 F 6, After stability study at 4ºC (45x)

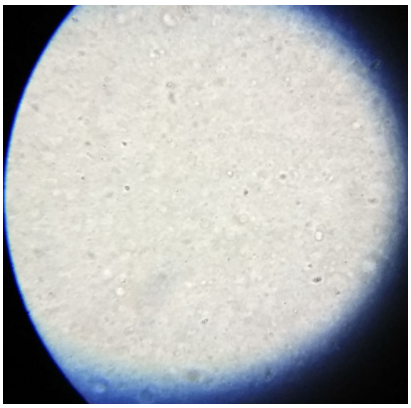


Figure No. 40 F 6, After stability study at 25ºC±2ºC (45x)

After one month, Metformin HCl liposomes formulations F 1 to F 6 were showed difference in *in vitro* drug release profile. Dissolution rate was decreased in all Metformin HCl liposomes formulations at both storage conditions like 4°C and 25ºC±2ºC. The results of *in vitro* drug release of all the formulations at both storage conditions were compared with before and after stability studies and the results were shown in **Table No. 10** and **Figure No. 40.**

Table No. 12 *In vitro* drug release data of all the Metformin HCl liposome formulations after stability study, compared with before stability

S. No.	Formulation code	Immediately after preparation	After stability study	
			At 4°C	At 25ºC±2ºC
1.	F 1	103.03±2.47	91.81	73.38
2.	F 2	91.92±2.72	86.77	68.26
3.	F 3	82.12±2.51	77.91	64.37
4.	F 4	100.58±1.12	91.74	87.41
5.	F 5	85.06±1.73	78.81	61.81
6.	F 6	79.05±1.03	73.98	63.32

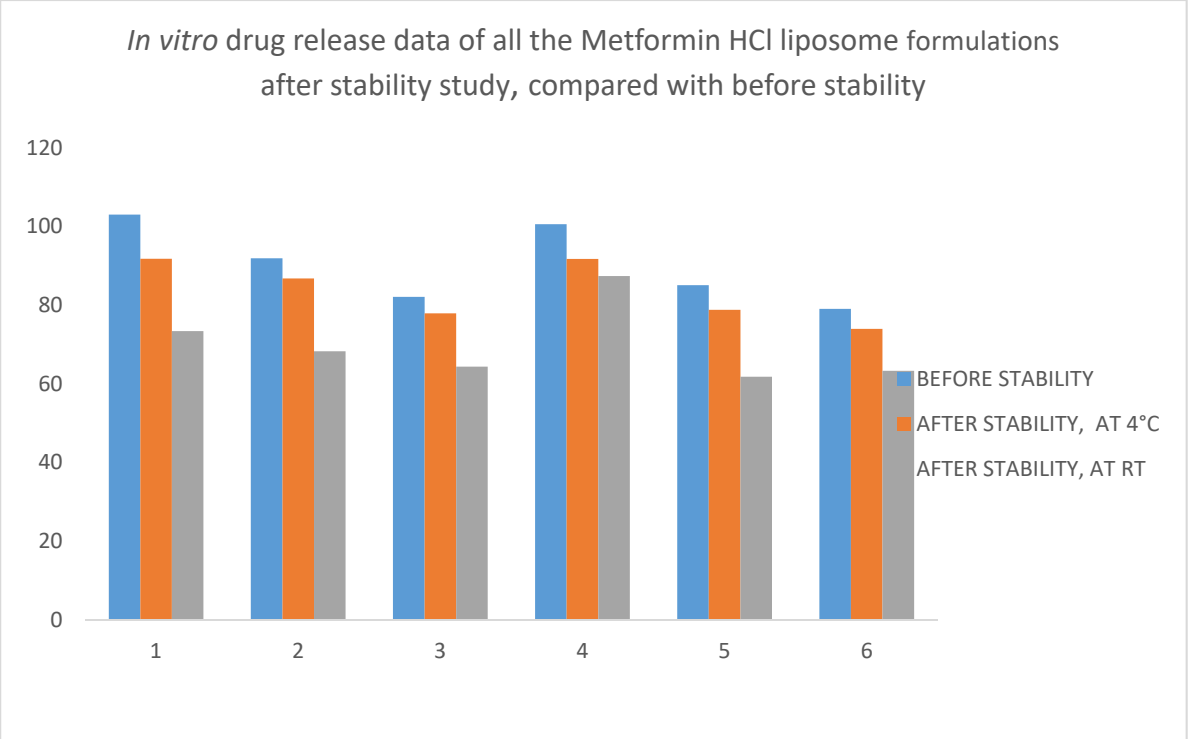


Figure No. 41 *In vitro* drug release data of all the Metformin HCl liposome formulations after stability study, compared with before stability

F 1 F 2 F 3 F4 F 5 F 6

At storage condition 4°C showed better stability than another condition. This may due to their elevated temperature reduce the stability. But in both storage condition higher proportion of soya lecithin contains formulations like F 3 and F 6 showed better stability than other their formulations.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

This study concluded that Metformin HCl was successfully prepared as a liposomal drug delivery system by using two different techniques such as physical dispersion method and ether injection method. In this liposomes preparations, cholesterol ratio was constant and soya lecithin concentrations were gradually increased (like 1:1, 1:2 and 1:3). The liposomes prepared by physical dispersion method showed better percentage drug entrapment when compared with ether injection method. The morphological characters of prepared liposomes were determined with the help of optical microscope. The particle size was analyzed by Malven particle size analyzer. The results of the particle size showed, when the concentration of soya lecithin was increased the size of the particle was reduced. The *in vitro* release showed that as the concentration of soya lecithin was increased the release rate of drug was retarded. Among the two methods ether injection method showed prolonged action when compared to physical dispersion method. The stability studies for all the formulations were performed by keeping the formulations at two different temperatures 4ºC±2ºC and 25ºC±2ºC for a period of 30 days. After the stability period the formulations were tested for morphological analysis, percentage drug entrapment and *in vitro* drug release and compared with before stability study. There was no change in morphological characters at 4ºC±2ºC, but there was a slight reduced in particles size at 25ºC±2ºC. The percentage drug entrapment was reduced in all the formulations at both the conditions. The *in vitro* drug release was reduced for all the formulations. Liposomes prepared by physical dispersion method showed better stability compared with ether injection method.

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BIBILIOGRAPHY

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